[MUSIC PLAYING]

MIGUEL REGUEIRO: Prior to bringing Rick up to discuss some of the basic mechanistic features of inflammatory bowel disease, we'll start at the bedside. And I really look at this as three components to the talk. One is to launch the data and understanding on how we treat Crohn's disease after somebody has surgery. The second is to give you a bit of some insight in how, maybe for the young investigators in the room, because I'm often asked about career pathways, how a very small study can turn very large. And for those looking for studies in niches, I'll try to sprinkle this with some career advice along the way. And then third and most importantly, is to launch the idea of this model as a potential for immune-mediated diseases and understanding immune-mediated diseases but really to launch what Rick Deurr is going to talk about from a basic mechanistic standpoint.

So I plan to provide a background on the clinical problem, talk to you about our post-operative study here at UPMC, but then also understand how the post-op study and the pilot that we launched really turned into a protocol in Pittsburgh. I know this is a fairly small niche, and for those that are outside of not only GI but specifically Crohn's and colitis, you've probably never heard of this. So I recognize that, and I'm very humble in knowing that probably less than 0.001% of the country knows what I'm talking about. So that's OK. But then I'm going to launch this, as I said, into Rick's platform.

So as a background and just to put this into context, what's inflammatory bowel disease? So these are immune-mediated gastrointestinal diseases that really span Crohn's disease and ulcerative colitis. So if you look at the left-hand bottom side, that's UC, ulcerative colitis, and the bottom right is Crohn's disease.

Ulcerative colitis is inflammation that occurs in the colon, usually starting in the rectum, and it goes up in variable degrees throughout the colon, but it's primarily a large intestine inflammatory process. On the right-hand side, Crohn's disease occurs anywhere from the mouth to the anus, and it skips around in the bowel, with the small intestine, what we call the terminal ileum being the most common area for ulcers and inflammation. We now know that there are different causes, meaning genetics, the immune system, and the environment, and again, Rick will talk about some of that in a minute.

We also know that for both diseases, but specifically on the right-hand side, Crohn's disease, that over time, if the body causes enough inflammation, there's structural damage in tissue that's irreversible and requires surgery.

So where do we stand today with Crohn's disease? About half the patients we still see require surgery at some point in their lifetime. What's happening in 2016? And I would say that this really applies to probably all diseases but specifically immune-mediated diseases, so like rheumatoid arthritis, multiple sclerosis, lupus, you can extract the disease that you want, but this is the model. This is the platform.

As a gastroenterologist, when we meet and see a patient who has Crohn's disease, they don't present silently. Usually, we're not doing screening colonoscopies on all children and seeing if they have some type of inflammatory bowel disease. So when do they present? They present when they see you as a primary care physician for a bowel-related symptom or possibly for a non-bowel-related symptom, arthritis, uveitis, iritis.

However, there are a certain percentage of patients that we see for the first time who already have irreversible damage and require surgery. It's not necessarily a failure of medicine. It's just they're presenting with symptoms beyond the ability for us to reverse it.

So we came up, not only our group here, but others across the world have realized for years that there's a post-operative Crohn's disease model. So let me just walk you through this, because I think as you think about the basic mechanism, this will become clearer.

So imagine you take a patient. You don't need to know anything about Crohn's, but imagine that 10 centimeters of their ileum is bad. It has Crohn's. It's ulcerated. It's strictured. It has holes called fistula, and they need surgery, all right? You resect that segment of their bowel. It's like pipe-- when I describe this to patients in the clinic-- you take out the bad piece of pipe. You hook the two good ends together.

Above that and below that, the surgeon, for a split second in the OR calls you, and says the patient's cured of Crohn's. They have no Crohn's disease. What do we know now though? Look to the right. First slide on the right is histology. We know that one week after a macroscopically normal surgery, bowel is resected, good ends are hooked together, if you biopsy the macroscopically normal tissue, there's already evidence of Crohn's in a high percentage of patients based on that biopsy finding.

No. We don't do colonoscopies one week after surgery. This is research protocols, but we found that tissue cells start to recur with Crohn's early. What happens when you go over to the right? You do radiographic testing or endoscopic testing and lo and behold, you see their ulcers occur.

But look at the top of the slide. Most of these patients are asymptomatic. The deepest remission we can do is take a patient with bad Crohn's disease, feels terrible, bowel blockages, pain. Resect that piece, they feel great, but if you look within the first year, you see a high percentage of those patients already have ulcers by one year.

Go further to the right, where you see the picture of the guy holding his belly. If you do nothing at all, about five years later, 2/3 of patients come back with symptoms, and then ultimately, what happens is they need surgery again. So this is the model by which we are now launching a number of studies, and Rick Deurr will talk a little bit about the microbiome genetics.

But this is also one immune-mediated disease where we know predictably where there's recurrence, and we know that we can remove that part of the bowel and start over. Can't do that with rheumatoid arthritis, multiple sclerosis, and other immune-mediated diseases. So this is launching a platform for microbiome genetic immunology research.

So this is the background. We're involved in some of these studies, but now I'm going to walk you through what do we do clinically?

So it's interesting. There came up with a scoring system years ago, and I'll just make this simple. One year after surgery, if you do a colonoscopy and you look at the bowel, we term it as endoscopic remission, and you don't need to be a gastroenterologist to understand this. You do a scope. You get to the anastomosis, the hook-up, you look in their ileum, and if you see zero ulcers, nothing at all, you give it a score of zero. If it's less than five little ulcers, it's a one. Recurrence is more than five ulcers, two, more severe inflammation, deeper ulcers, three, and a four is ultimately the patient has more stricturing disease or severe disease.

When you have a patient who feels well, may not have symptoms, but gets to that three or four, often they'll need another surgery within five years.

So these are just some photos, examples of some of the early studies looking at what we call an endoscopic score. On top, that just gives you an idea of what one of those little ulcers looks like. So for the non-gastroenterologist, that's an aphthous ulcer in the ileum, and the bottom left is more severe inflammation ulceration. We'd score that a three, call it recurrence. And then again, you don't need to be a gastroenterologist to see at the bottom, right, it looks pretty bad with some narrowing.

So what's the algorithm for treatment for post-op management? And this is how, up until about 10 years ago, we looked at post-op disease. We had mesalamine, which are anti-inflammatory, antibiotics, steroids, and then the immunomodulators, so 6-mercaptopurine, azathioprine, so 6-MP Imuran. And the question is, and this is where we came into play about a decade ago or a little bit longer, and we said, can we reverse this process of recurrence? You know, these medicines that we're using, are they the best? And the biologics hit the market, and we saw an opportunity to maybe explore and expand the horizons in understanding.

So I'm just going to present two cases that you may see in your medicine clinics or some of you that aren't in GI but in rheumatology or other diseases. So these patients present with all types of systemic manifestations that probably click off at all of the disciplines within department of medicine.

So this is a 32-year-old smoker, Crohn's, only been on mild treatment with Pentasa, that's mesalamine or 5-aminosalicylate and has had about one to two times per year, steroids. Now, comes in with a small bowel obstruction. So we're not radiologists. So just follow the arrows. This is the radiographic sign, the yellow arrows. This is a terminal ileal stricture, and the patient's starting to get an obstruction, dilation of the bowel above that.

So the patient comes, and they get surgery. 10 centimeters of their ileum is resected. They're connected back together, and the question now, the patient comes back, and they say, you know what? I feel great. This is the best I felt in five years, and they're asking you, what do we do at this point? How do we prevent this from happening?

But look at the phenotype. Look at the flavor of this patient. That's very different than this patient. 43-year-old Crohn's comes in with severe pain and a fever. Not been on any medications. The diagnosis is new. Smoking, yes, you see a pattern. Smoking's bad for Crohn's. Bizarrely, and it's probably carbon monoxide, but smoking may be good for ulcerative colitis. So we have to look to our pulmonology colleagues to do some of the basic CO mechanistic work, but maybe that's why smoking helps ulcerative colitis, but that's another talk.

So she smokes, she has Crohn's, she has bad disease. And a CAT scan on her is performed and not like the last guy, who has a stricture, this patient has pretty bad disease, a lot of inflammation, phlegma, and fistula and an abscess. She gets the abscess drained. She comes to surgery. Ultimately, comes back. She has a fistula, and the patient also tells you she feels quite well.

So one phenotype, stricturing disease, another phenotype, fistulizing disease. So you're seeing a pattern that Crohn's disease, like all diseases, are not just one disease.

So in 2009 and before that, we decided here to put together-- we did a network meta-analysis, but I'm just showing you the simple table without the full analyses. And we looked at all of the medicine trials, and I have 300 slides to follow this, and I'm just going to show you one. So don't worry. I'm not going to show you 300 slides.

But what we did is we sat down, and we looked at all of the data, clinical recurrence and then, in the middle column, clinical recurrence, meaning symptoms come back. endoscopic recurrence means you see the Crohn's come back when you do a colonoscopy. And we looked at the medicines, placebo-controlled studies against five ASA budesonides, a type of steroid, nitroimidazole antibiotics, those are metronidazole or in Europe, they use ornidazole. We don't have that, or we don't use that in the United States. And then finally, the immunosuppressants, azathioprine and 6-MP.

Don't have to be a gastroenterologist, but probably one box strikes you, and I'll come back to that in a minute, when you look under clinical recurrence and antibiotics. But also one of the things that strikes you is when you compare to placebo with the endoscopic recurrence, the top, right box, endoscopic recurrence with placebo, and you compare it to other studies under network meta-analyses, you did not see a statistical benefit of most of the treatments over placebo. So that was a little bit disconcerting to us, because I told you a minute ago, if the patient gets ulcers and we can't heal them, a lot of these patients need surgery again.

So let me digress for one second. Look at the nitroimidazole box under clinical recurrence. That had the lowest rate, and this was across trials, so three or four trials. What does this tell us? So I submit to all of you in the room, if you have an interest in the microbiome, there is something about the microbiome that's very real, not only in Crohn's, but probably all immune-mediated diseases, but we see it very clearly in the Crohn's disease post-op studies, the lowest recurrence rates. The problem is you need to take one to two grams of metronidazole a day, and you can't stop.

How many of our patients drink alcohol? Well, most of mine do. So they won't listen to me and stop drinking alcohol. How many of them have a problem taking high-dose antibiotics? Most of them. So if we find the microbiome-altering agent or process-- and yeah, it might be diet-- but the process, this will be ultimately the treatment for IBD.

So we looked at those studies, and we said, you know what? This is strange. And I was looking for something to do, because I didn't have a niche, and I said, you know, this is probably not the best approach. And the question is, can we do better than this? So this is where we came up with a proof of concept study. This, by the way, was not meant to be the pivotal end study. This was just a proof of concept investigator-initiated study that we looked at here.

And we said, great. We'll do a randomized, two-arm, double-blind, placebo-controlled study, and we'll assume-- and we compare this to infliximab, anti-TNF. We'll assume in the placebo rate, if we didn't do anything to the patient after surgery, 80% would endoscopically have recurrence. That's been proved. We've seen that in multiple studies.

We had no idea with an anti-TNF what the post-op rate would be, because nobody's done that before. So I asked my statistician, give me a low number that makes sense that we can power to get a small number of patients at our site here in Pittsburgh. He said fine, 20%. Literally, that's what we ended up doing.

So we enrolled 24 patients, randomized half to infliximab, anti-TNF and half to placebo for one year. By the way, for the young investigators in the room, I went to three senior researchers in IBD in the world and presented this study and all three uniformly told me this was the worst idea that anybody has ever had. It will never work. It will never get published and start thinking about doing something else with your career. And I mean, it was pretty harsh, and one of them said that, literally, and I see him to this day and laugh. But nonetheless, that's fine. But the other two said, this is a bad idea.

So for the young faculty investigators out there, if you have an idea that clinically makes sense and there's science behind it, follow your heart. Do the study. And especially if you have-- in my case, we had some outstanding surgeons who said, you know what? We're going to collaborate. So I didn't go within the Department of Medicine. I went outside to a pathologist and a surgeon, and we came up with this protocol.

What did we find? So endoscopic recurrence at one year, placebo's in red and yellow is infliximab. Yes, it's only 24 patients. Yes, it's very small, but we saw a strong signal. We saw a difference in that the placebo patients, as expected, 85% had recurrence endoscopically, where in our anti-TNF group, we only had one recurrence. Again, small study, not meant to be a be all, end all study.

We also did separation of scores, which I won't go into too much detail. But the bottom line is, if you look on the far left, that's a score of zero, totally normal. Far right, three and four scores. Remember, those are the scores I showed you that are severe recurrence that lead to another surgery.

But this is also for the young faculty but more specifically for the residents. This is a study of 24 patients that we had a lot of questions, and we had a great data set. And even though it was just 24 patients, we started asking other questions. So first, we published this in *Gastroenterology.* So this came out in 2009, and then this led to other studies.

So we said, does our activity score, Crohn's disease activity score, apply to post-operative Crohn's? Nobody's really looked at that. And I said, all right, fine. Maybe we can look at what our Crohn's disease scores were that first year and see if it correlates with endoscopy. So we published that, and we found that no, the Crohn's disease activity index does not predict endoscopic recurrence, and we published this, then, in 2010.

We also wondered if giving a biologic immunosuppressant immediately after surgery is safe. Our surgeons were terrified of this. They're saying, what do you mean you're going to give somebody two weeks after an intestinal resection with a microbiome going crazy and the belly just having an abscess a potent anti-TNF? So we wanted to know was this going to create a problem, and we found that it did not. We actually found that giving anti-TNF shortly after surgery did not lead to more side effects, and we published that in 2011.

And then finally, out of this 24-patient study, we looked at, great. Well, Crohn's diseases isn't a one-year disease. It's a lifelong disease. Does this recur long term? So we've now followed our patients for, well, now we have over 15 years of data on these patients, but we followed our patients for eight years. And we published this in our *Clinical Gastroenterology and Hepatology Journal,* and we actually found that people who stayed on anti-TNFs did better than those that did not. I will also tell you that we don't treat everybody after surgery with an anti-TNF, and I'll come back to that in one minute.

But I've debated many people at national, and some of you that are Fellows have seen me debate at these national conferences and say, how can you use these data to make such a statement? And one of the guys I debated came up with the Pittsburgh Post-Op Bus. So I'm borrowing his slide, and he put me as the driver. So this was used against me in a talk, but I'm using it for me right now to say that a 24-patient study, which is what we had here in Pittsburgh, led to bigger studies.

So what did it lead to? This is it. 10 studies came out of this. Not at our site, these are international studies with different, two different anti-TNFs. And if you look at the middle column, anti-TNF, the rate of recurrence is about zero to 20%. So my statistician telling me it was about 20% recurrence, he should have played the lottery, because that's what we started seeing. It's about a 20% recurrence, and we've seen that across countries, across two different anti-TNFs and across studies.

But then, I've had the privilege recently to lead a very large international study called the Prevent Study. So this included over 365 patients. We've had every continent that's been enrolled patients, and actually this is timely, as this was just published last week in *Gastroenterology.* And the results from this post-op study, if you just look at the left-hand side where gray is placebo, orange is infliximab, anti-TNF. Again, you see that rate. For the gastroenterology Fellows, remember 20%, 22%, that's the endoscopy recurrence after using anti-TNF, and this was statistically better than placebo.

So this also led to a number of other reviews and treatment. So for the mentorship part of this talk, go with an idea that makes sense that you see in a clinic that also makes sense scientifically, and don't give up on that idea.

So this is now called the Pittsburgh Post-Op Treatment Algorithm. And this is, we've been-- we've actually published this in over 10 of our journals, and I'm seeing this now picked up in others, where we give a phenotype and disease behavior description to the patient after surgery. Are they low risk, medium risk, and high risk?

So low risk group of patients would be that patient that you see, who's had Crohn's for 50 years and comes to their first surgery. Would I put them on a biologic post-op? No. The natural course of disease suggests that this is a very benign course. We leave those patients alone. We do a colonoscopy a year later and only if they have recurrence, we treat them.

The medium risk group are those patients that come to their first surgery within 10 years. And no, we don't go to a biologic. We use an immunomodulator, and remember what I said about the microbiome. And remember the challenge that I have to all of you. If you can come up with a microbiome-altering agent, that's not metronidazole, which is still what we use, but patients just don't tolerate it long-term. That may actually be the treatment for that medium risk group.

And then the high risk group, multiple surgeries, fistulizing disease, somebody with very severe, early onset, kids that get Crohn's that get multiple surgeries. That's telling us phenotypically and based on probably their immune-mediated profile, something's different about them. That's the group of patients that we consider anti-TNF.

So I'm going to end with the last couple of slides before I turn it over to the bench, who will give you all the answers on why this all happens. I just show you what we see at the bedside, which is nice, because then I can sit down and let Rick explain it all. But what's next for the Pittsburgh Post-Op Study?

So again, I've had the fortune of leading an international group. We actually just put together an American Gastroenterology Association technical review and guidelines. We just submitted it. It was approved. It goes through-- for those that are involved in international guidelines, there are a series of reviews, the peer review-- or sorry, the patient review panel just approved it. So this should be coming out in the next six months. Where we've looked at all post-op Crohn's from every angle, and we did PICO to come up with some specific questions and large network meta-analysis as well.

We're also looking at different molecules. So remember, this is an immune-mediated disease that we know where it recurs. So there's an alpha 4 beta 7 integrin. We just got approved here. Jason Swoger, my colleague, is going to be the principal investigator on that. We're going to look at that mechanism of action in post-op Crohn's. We were just approached recently with an IL-12/23, Rick Deurr had done a lot of the basic genetics work on this, and there's going to be an agent that we're looking. And just this morning, a small molecule group, looking at oral small molecules, which are going to hit the market for us. They're out for psoriasis now, but we're going to start using it for IBD, will be applied as well.

And then Johnna Hashash, my clinical faculty colleague and Olia Finn, who many of you know in immunology are looking at Muc-1 vaccines, and we're going to apply a Muc-1 vaccine potential trial. We're looking at Muc-1 mechanistically, and we just submitted this for a large research grant as well.

And then finally, like I said, the microbiome and genetics are the key, and I think Rick Deurr has an exciting study that's come out of this model.

So don't give up on a small idea. Know that these are sometimes the best ideas and this leads to a number of other research. The question is, and I'll leave Rick with this, how much is genetics? How much is the microbiome? And where does the immune-mediated factors play a role? So thank you very much.

RICHARD DEURR: So I'd really like to thank Miguel for doing a great job setting up my talk. And if you take nothing else home from this talk, there is really three points, three take-home points. Number one is Miguel has laid out nicely, the inflammatory bowel diseases are heterogeneous conditions mechanistically, probably, that have some very similar clinical features. But in order to bring personalized medicine someday to the population, we really need to understand those mechanisms. And the way to do that is, I think, multi-omic data integration.

You know, the second that I want to make is that in order to enable multi-omic data integration, each of us that are in the microbiome silos or in the genetic silos need to, while we're collecting samples, make sure that we get the other samples. So, for example, if there was going to be a big project to collect microbiome samples, we should take advantage of that opportunity to also get some genetic material in, because it's going to be important to integrate it all.

And then the third is that this clinical model that Miguel has presented, post-op Crohn's disease, I think, is a wonderful, wonderful model for us to study all these different things that are going on mechanistically as Crohn's disease recurs post-op in front of our eyes. And so those are the take-home messages.

All right. So this slide attempts to summarize what we know about the different components that contribute to IBD pathogenesis. The thinking really is that in many patients, IBD results from an aberrant immune response to resident gut microbiota and/or other environmental triggers in the genetically predisposed host.

I'll spend a little bit of time talking to you about what we know about genetic susceptibility. The take-home point from that is that we have about 200 loci that have been discovered in genetic mapping studies, and these have implicated specific biological pathways in IBD pathogenesis.

We also know that the microbiome composition is altered in patients with IBD. They clearly have reduced diversity, at least for bacterial component of the microbiota. It's not clear yet whether other microorganisms, like fungi or something, fill the void of reduced diversity. And there are certain families of microbes that are either increased or decreased in IBD patients compared to healthy patients.

Now, we don't know what came first. We don't know whether the microbial changes came first or whether they're a result of the chronic inflammation, but I think we're going to be seeing that it's probably a mixture of both, and I'm aware of some emerging data that would suggest, in fact, that there are predisease changes in the microbiome. And it's very attractive to think that the genetics that influences the innate immune response and the adaptive immune response, that protects the host against pathogens that reside within the gut, could, in fact, change the composition of the microbiota. And then the metabolome of the microbiome could-- metabolic products from the microbiome could change and then feedback and influence the host.

And I don't have time to talk about it today, but we're in the process of trying to get a really interesting study published, where we identified a new IBD-associated variant with a chip that was designed to screen low frequency coding region variants. And we found a new variant, and when we looked in the literature, this particular polymorphism was associated in previous studies with a lot of seemingly disparate phenotypes, like anything from obesity to lipid levels to schizophrenia. And when we looked through the literature, the only thing that seemed to perhaps tie all those different phenotypes together was perhaps microbiome changes.

And so, in fact, we've now been able to show, and we're trying to get it published, that it looks like this particular genetic variant can, indeed, influence microbial composition. And we've been able to show that there is sharing of reduced OTUs in Crohn's disease and in obesity that's statistically significant. And the idea is that perhaps those changes are predisposing to both of those seemingly disparate phenotypes.

Environmental triggers, I won't say more about today. And the immune response, you know, certainly, it's been known for a long time that T helper 1-mediated immunity is up in IBD. Since Th17 cells have been recognized over the last several years, we know that those are also playing a role. And we know, in part due to the genetic clues, that there is defective innate immunity.

So the next couple of minutes is going to be just a bit of a humbling experience for me, and that's because everything essentially that I've done with respect to genetic mapping in the last 20 years can be distilled down to this slide and the next slide.

So yeah. So basically, this slide here attempts to summarize the results from a project, a very large project that was done in tens of thousands of European ancestry patients with IBD and healthy controls as well. It was part of the Immunochip Project. The immunochip was a chip that was designed via a collaboration between multiple different immune-mediated disease consortia, who had all done their own GWAS, and then through that process, had realized that a lot of the signals, a lot of the genetic association signals that were being discovered were shared across a lot of different immune-mediated diseases.

So all these things consortia came together, designed the chip together, and we put on the chip most of the promising hits from all the different GWAS that had been done in these immune-mediated diseases, and we also put onto the chip very dense maps of SNPs in the loci that had already been established. And then this chip was used to genotype in IBD and psoriasis and rheumatoid arthritis, you name the immune-mediated condition, it was used. I think Illumina, that produced this chip, made a lot of money off of it.

But this slide summarizes the results in the Caucasian IBD part of the Immunochip Project, and the top half of this slide is a Venn diagram that really tries to get across the message that of the about 163 loci that were discovered in European ancestry IBD with the GWAS before this chip and then this chip, there's really kind of a continuum of risk of the alleles that were discovered, from Crohn's disease to both forms of IBD to UC.

And that even though in the middle, for example, we were showing that 110 of the loci that we discovered with this chip and all the studies before it, confer risk for both CD and UC, there is, for at least some of the loci, a strong bias towards one phenotype or the other. And even for the loci that we say are Crohn's disease-specific or ulcerative colitis-specific, on the left and right sides of the slide, there's a significant trend for many of them towards the other phenotype as well. So this gets to the idea that IBD shares a lot of risk loci, and, in fact, IBD shares risk loci with a lot of other immune-mediated conditions.

So the bottom half of the slide attempts to show the variance explained by these loci. And the total variance explained by the CD loci that we knew about at this time, back in 2012, was 13.6% of the variance and for UC, it was 7.5%. Now, that's going to go up, as we get closer to causal variance. There's a lot of additional sequencing studies that have been done, and as we do this multi-omic data integration . and go from just tag SNPs on a chip to causal variants, that will go up. But this shows what we know as of the time this chip was done.

And the proportion of the variance explained is shown in the bars below. The width of the bar is proportional to the proportion of the variance explained. The bars are connected between CD and UC if there are shared loci across those two phenotypes.

So a couple of things here, you can see for Crohn's disease, the Interleukin-23 bar locus that we discovered in 2006 is a biggie, as is NOD2, that was actually discovered before GWAS were even done by family linkage studies back in 2001. And for ulcerative colitis, the MHC stands out.

So the MHC is a big factor in many diseases but interestingly, it doesn't play that much of a role, proportionately, in Crohn's disease. So UC and RA, and psoriasis, and everything else, many of the immune-mediated diseases have a big, big MHC effect. That's not true in Crohn's disease. The other locus that's a big player in ulcerative colitis is on chromosome one, and I won't be able to talk about that today.

I am going to say a little bit more about the variants that are circled here in violet and some of the others associated with them, because they all have something to do with the Interleukin-23 signaling and the Th17 or type 3 ILC pathway.

The Immunochip was also used to genotype non-European ancestry populations more recently, and in particular, there was a large East Asian population that was genotyped. And this slide actually is from a study that was published, the Immunochip Experience in a now larger Caucasian group of study subjects plus East Asians that were genotyped, and it makes the point that, in tan, a lot of the loci are shared across these ethnic ancestral groups, but there's also some differences. So, for example, for Crohn's disease, you can see in blue, that NOD2 and one of the major Interleukin-23 receptor loci that's strongly associated with Crohn's in European ancestry is monomorphic in East Asians.

And then for some of the loci that are shared, there is much, much bigger effects in one population or the other. So, for example, you can see for the TNFSF15 locus, which is very important in TNF effects, it's a much, much bigger effect in East Asian than it is in European ancestry. So I mean, I think that these population differences, again, speak to heterogeneity. There's going to be different effects of genes in different populations and probably lead to some different clinical aspects and mechanisms of disease as well

All right so that's all I'm going to say about gene mapping, even though, you know, that's what I did for the last 20 years, and I'd like though, to look forward, beyond GWAS to how we're going to translate all of this information from the GWAS into something that's clinically useful. And I think that the way that we're going to answer those questions is going to be challenging, because we know that a lot of genes are implicated by these GWAS signals, more than 4,800 reside within the 163 regions found in the European ancestry.

GWAS, we know that there's a lot of these signals that could be described by multiple different SNPs that are in strong linkage to disequilibrium, and therefore, you can't use genetics to separate the effects of one from another. And so it's hard to know, in many cases, which SNP is actually, from genetic studies alone, causative.

And the other thing that's challenging, I think, for us, is that most of the genetic variants with the strongest effect sizes are in intergenic or intronic regions. They don't code for protein changes, and so they probably have something to do with regulating gene expression.

So what are the approaches going forward? As I alluded to at the beginning, it's really, I think, multi-omic profiling, gene expression profiling, epigenetic profiling, looking at gene/microbiome interactions and then putting it all together in multi-omic data integration approaches. And I think this particular study that I'm showing you one figure from is really a promising study that there's a light at the end of the tunnel.

This study was published in *Nature* last year, and basically what these investigators, Farh, et. al did is they took all of the GWAS signals, all the GWAS data that were available at the time, and they tried to distill all the genetic association data down into a set of likely causal SNPs by doing the best they could to use genetic association signals to limit the number of variants that are likely to be causal.

They also studied a bunch of different cell types, 33 different cell types and did kind of epigenome-wide screening for different epigenetic marks. And those are-- and the cells that they screened are all listed in the vertical axis. And then they looked for enrichment of the GWAS signals for 39 diseases, listed across the bottom, horizontally, in these histone 3 lysine 27 acetylation regions that are markers for active promoters and active enhancers.

And interestingly enough, they found some signals that really make sense. So in the very top, left part of this slide, you can see that there's some signal going on in neurological phenotypes, Alzheimer's disease, progressive supranuclear palsy, in cells that come from the nervous system. And then you can see kind of just below that, a bunch of immune-mediated diseases listed. Is there a pointer here? I guess not.

But anyway, there's a bunch of immune-mediated diseases listed and there's enrichment for immune-mediated disease signals in immune cells and so on. And the columns that are pertinent to our phenotypes, Crohn's disease and ulcerative colitis, are demarcated by the two vertical red lines and the take-home message from this heat map of candidate causal SNP enrichment in H3K27ac regions for Crohn's disease and for ulcerative colitis is that there's enrichment of immune cell signals for both Crohn's disease and ulcerative colitis in these H3K27 acetylation regions. And in UC, there's enrichment for GI epithelial cells and that's not true in Crohn's disease. So I think what this does is it points us to-- this kind of information can point us to the tissue types and cell types in which we should be doing our functional studies.

OK. So this is a pathway that is one of my favorite pathways, because we found the Interleukin-23 receptor association back in 2006. And both Crohn's disease and ulcerative colitis, we now know, share a lot of the genetic association signals that are relevant in this pathway. And I've highlighted in red some of the genes encoding proteins that are relevant in this pathway. From Interleukin-23 locus, the Interleukin-23 p40 subunit of IL23, TYK2, JAK2 signaling, STAT3, et cetera.

And this pathway, this IL23 signaling pathway, is important for the development, maturation, and maintenance of Th17 cells and also for type 3 lymphoid cells, both of which are probably very important in mucosal homeostasis. And when something goes wrong with those cells, we could potentially get chronic intestinal inflammation.

The other thing though, that's been realized in recent years is that these Th17 cells don't necessarily have a stable phenotype. We oftentimes think in immunology that the effector cell, once committed to be that type of cell is committed, and it doesn't change its phenotype. But Th17 cells, we now know, are quite plastic. And depending on the environment that they're in, they can morph, for example, in this example here, under states of chronic inflammation, from a classical Th17 cell that makes IL17 through to a cell that's a hybrid cell that looks a little bit more like a Th1 cell, and finally, can morph into something that doesn't even make IL17 anymore. It makes interferon gamma.

And I haven't shown here, but these Th17 cells can also morph, depending on the environment, into regulatory cells. And what's important is that a lot of the genes that are thought to be important in this process are implicated in IBD GWAS, and so you want to figure out what's going on there.

And to do that, we have been doing a little study that attempts to integrate information about chromatin accessibility and gene expression in a subset of effector memory T cells that bear a surface marker CCR6, which is enriched for both Th17 cells as well as T regulatory cells. CCR6 is probably the surface marker that's important for cells to go to sites of tissue inflammation in the periphery.

And what we've done is we've isolated these cells from peripheral blood, not from lamina propria yet of gut, but from peripheral blood, and we've stimulated them under various conditions in ex vivo cultures that include different combinations of Interleukin 1-beta, Interleukin-23, and Prostaglandin E2. The reason we chose those three inflammatory molecules is that all three of them, we know from basic studies, are important in the process of development, maturation, and maintenance of Th17 cells, and all three of those molecules have receptors that are encoded by GWAS hits in IBD.

And so what we've done is we've, after isolating them, put them in short-term cultures under these stimulation conditions, and then we've done both ATAC-seq, which I'll describe in a second and RNA-seq in the paired samples. This just shows our gating strategy, and I won't go through this for interest of time. But basically, we're using common surface markers to sort these cells out after pre-enriching for CD4 cells.

We're also studying a marker, in addition to the ones I mentioned, called CD 146, because it was reported in other studies to be a marker of cells that go up in the circulation with flares of IBD. And so we wanted to track that as well, even though it's on a fairly small proportion of cells. This just shows that our purity after sorting is pretty darn good.

Now, this is a slide that basically shows the idea behind using ATAC-seq, which stands for the Assay for Transposase Accessible Chromatin with High Throughput Sequencing, to use that method for assaying open chromatin, to find out where in the genome open chromatin is. The reason why we want to know that is because open chromatin sites, sites where nucleosomes are depleted is where gene regulatory elements sit. And so we want to know which of our SNPs from the IBD association signals are sitting in gene regulatory elements? And then we also want to know, from RNA sequencing, which nearby genes have differential expression in the conditions that we've chose?

And so basically what the ATAC-seq does is it takes advantage of a transposase that inserts adapter sequences for lumina sequncing right in regions of open chromatin, and then it cuts those regions at the same time, and you can PCR these fragments up and then sequence the ends of them. And then you look for the where the reads pile up against reference and that tells you where open chromatin sites are.

And the reason why we're using it in many-- and why it's really taken off is because you can do it with very small numbers of cells. For example, the alternative to get similar data is DNAse 1 hypersensitivity sequencing, but you need lots and lots of cells and much more chromatin to be able to do that. So ATAC-seq gives you the same kind of information with fewer cells, fewer amounts of starting material.

And basically you just spin down nuclei. You do this transposase reaction, you amplify the fragments, you make a library, you QC it. We actually do size selection, because you end up getting a big broad range of fragment sizes that would be hard to dial in sequencing on the next generation sequencer if you don't size select. And then basically, you sequence and do all the standard QC and mapping, et cetera.

This is what a library looks like, an ATAC-seq library. And the notable feature here is that you can see these little humps that correspond to nucleosome periodicity, which is exactly what you should see if the enzyme is cutting in open chromatin regions. And this is what it looks like after, on a bioanalyzer after we've done size selection.

And with the help of Jason Lieb at the University of Chicago and Terry Furey at University of North Carolina and some bioinformatics and genetics collaborators here, we put together a good pipeline for analyzing these data, that I won't go through in much detail, but I can just tell you it works really well.

And this is an example of one of our tracings. We just finished doing about three dozen pairs of samples. And this is a region where we saw, in red above, a lot of pileup in one of our conditions that was significantly greater than in another. And then below, you can see from the human genome browser at UC Santa Cruz, that these peaks that we found above are aligning where there are known H3K27 acetylation sites and known DNAse 1 hypersensitivity sites. So the method works.

And we're in the process now of analyzing RNA-seq data, which has all been generated, and we want to integrate all that. We're hoping, then, that by integrating that along with SNPs in these individuals, we'll be able to get some clues about how the SNPs that map to the open chromatin regions influence the expression of genes that are nearby and are differentially expressed

And our promising, very promising early results just from the ATAC-seq data alone is that we are indeed seeing enrichment of IBD associations in these open chromatin regions. And so I think this is going to give us a lot of clues about where to go from here.

And then I want to just come back to the clinical model that Miguel introduced at the beginning. This is a slide that kind of shows a study design for a long-term project that we're involved in here in Pittsburgh. It's being done by the NIDDK IBD Genetics Consortium, and essentially, again, the idea is that if we enroll patients who have undergone a recent ileal resection, they're now post-op ileal resection patients, at that very moment in time, they don't have any macroscopic disease. And then if we follow them forward serially, and we collect biopsies and clinical data, and blood samples to measure different omics measurements and put it all together at the end, we may very well be able to get some clues about what actually the early mechanisms of recurrence of disease are and about heterogeneity.

So that's a long-term project that we're doing. We don't really have too much data yet. We did do a pilot study of the microbiome and indeed, in patients that are recurring early, it looks like they have reduced microbial diversity in comparison to the patients that aren't. But it's still too early to say anything definitive. We don't have any of the RNA sequencing data back or anything. We're probably about a year away from recruiting the last of about 300 patients that we've targeted, and then we need time for the follow-up colonoscopies that we're doing as well. So this is going to be a long-term project but hopefully, will be revealing.