Dr. Wapner: In prenatal diagnosis, it used to be identify the phenotype, ask the question, “What are the diseases that can cause this phenotype?” And, then test for those specific things that you could test for. And, when I say identify the phenotype I'm talking about by ultrasound. It's changed completely, because we now have the ability through screening or for other techniques, identify the genotype first and then we can use the ultrasound to evaluate the evolution of the phenotype. It's a total 180-degree switch.

Narrator: Welcome to the Illumina Genomics Podcast where leading scientists discuss their genomics research, and how genomics is shaping their understanding of science and nature. Here's your host, Paul Bromann.

Paul: Hello there! Thanks for joining me for episode 31 of the Illumina Genomics Podcast. There are several prenatal screening tests that obstetricians can use to estimate whether a baby is at higher risk or lower risk of having certain conditions. For example, a 1st trimester maternal blood test and ultrasound exam, called a nuchal translucency screening, can estimate a child’s risk of having a chromosomal disorder like Down Syndrome. A 2nd trimester quadruple marker blood test can help screen for chromosomal abnormalities, or aneuploidies, like trisomy 21 or 18. Cell-free DNA prenatal screening, sometimes referred to as non-invasive prenatal testing or NIPT, has also been developed to screen prenatal aneuploidies. In NIPT, a maternal blood sample provides maternal DNA as well as DNA from the pregnancy that can be analyzed using next-generation sequencing, or NGS. With all of these choices, how do obstetricians determine the types of prenatal screening most appropriate for their patients? How do patients decide which prenatal screening tests, if any, are right for them? And, how do prenatal screening tests like NIPT fit in the context of prenatal diagnostic testing? To help sort all of this out, we’re joined by Dr. Ronald J. Wapner, Vice Chair of Research in Obstetrics and Gynecology for Columbia University Irving Medical Center and Director of Reproductive Genetics. He’s an internationally known physician and researcher specializing in reproductive genetics, and he pioneered the development of chorionic villus sampling, or CVS. Dr. Wapner, thanks for joining us on the podcast first of all, I know you're really busy. And I've read that you've been doing this, you've been active for over 30 years.

Dr. Wapner: I have been, yes.

Paul: You've probably seen a lot of developments in reproductive genetics. So from a comprehensive point of view, how is prenatal genetic testing developed over those 30 years? What was the standard of care when you started practicing up until this moment? How has the field evolved?

Dr. Wapner: There've been an immense number of changes. Just to start, it was about 50 years ago when there was an observation that you could take a sample of amniotic fluid and that you could look at the chromosomes in the fluid. About that same time, we realized that extra chromosome such as that associated with Down syndrome had health consequences. Then there was some discussion about what to do with this capability, and the NIH had a consensus meeting and made the decision that the risk of, use the knowledge that the risk of certain chromosome abnormalities like Down syndrome

along with trisomy 13 and 18, increased with the age of the mom. There then was a discussion of how we would screen the population for this.

Paul: And this is 50 years ago?

Dr. Wapner: This is about 50 years ago. Yes. The discussion had a number of factors. The risk of having a child or fetus at the time with a chromosome abnormality increased with maternal age. But it wasn't a sudden increase at any age. As a woman aged, her risk became higher. So first of all had to make a decision of how old a woman should be before we offered her the opportunity to have prenatal testing, which at that time was done by amniocentesis. Amniocentesis had also just really been investigated, and the ultrasound hadn't even been discovered. So the early amnios we're done blindly, so one had to evaluate the risks of the procedure which were felt possibly as high as one in 100 would lose a pregnancy because they had a procedure performed. There were not many labs that even were doing karyotyping, so we couldn't offer this to everybody because the resources were limited. So the decision was made that a woman who is 35 or older at the time of delivery had a high enough risk of having a pregnancy with chromosome abnormality to warrant doing this new diagnostic test which was called amniocentesis. The risks of which were still being investigated.

Ultrasound came along, and we were now able to see where the heck we were putting the needle in. So the procedure became safer. But still, approximately five to 15 percent of women having children during that period had been 35 or older. That number has increased, so it's now closer to 15 percent. At the time it was much lower. So we were doing or offering amnios to approximately five to 15 percent of women.

The question then became twofold. Number one, is there some way we can perform procedures on less women? And number two, we were only identifying women at risk who were 35 or older, yet although they had the higher risk, there still were down syndrome pregnancies occurring in younger women. About that time, we realized that there were blood markers or difference in hormone, or endocrine, or protein levels in the blood of some women carrying children with these chromosome abnormalities. And then we began to take advantage of that, and screening then changed from screening by just asking how old a woman was and that would be her screening test, to then being able to add her age to levels of biochemicals, to some ultrasound findings, et cetera. So that matured over time as the markers became better. So, in the beginning when we were offering procedures to just women 35 or older, every woman that had an amniocentesis only had about a one in a 100 probability that that fetus was affected, and we only identified 30 percent of affected cases.

As we add markers, we were able to decrease that number so that a woman's risk became as low as one in 20 to 1:25. But by that I mean that every woman that elected to have a diagnostic procedure had about a one in 25 risk that the fetus was affected as opposed to one in 100 when it was age alone. We also were then able to increase the number of affected pregnancies from as I said, 30 percent with age alone. But with the best in biochemical and ultrasound markers, we now could identify 70 or 80 percent.

So Over that time we've been much better able to identify the couples that were at risk for having an affected pregnancy. The other thing that happened simultaneously was we now had chorionic villus sampling, whereas amniocentesis was done around 16 weeks and in the beginning, it took four or five weeks to get results. So women were halfway through their pregnancies before they were getting information. With CVS or chorionic villus sampling, which samples the placenta in the first trimester, we now could get results by 12 or 13 weeks, which made a gigantic difference in patients' reproductive options if they had an abnormal pregnancy. More importantly, we could reassure women everything was fine much earlier. That was what was going on while we were doing karyotypes and looking at chromosomes under the microscope. The next revolution was the introduction of molecular cytogenetics. So rather than looking at the chromosomes, we were able to break them into small molecular pieces and do things like microarrays. With microarrays, we were able to identify much smaller missing or overrepresented pieces, microdeletions and micro duplications.

While that was very interesting from a laboratory standpoint, but we then had to figure out what's the clinical implications of that. In the clinical genetics arena, once they could do that, they found that there were a lot of children who had previously unknown causes of developmental delay, dysmorphic features, etc. And about 15 to 20 percent when they were tested for these sub microscopic findings, that was found to be the etiology. So we began to recognize sub microscopic syndromes and sub microscopic causes of developmental delay and problems like that. So now the question became, should we introduce this into the testing paradigm when you do amnio or CVS? So there have been studies that have now shown that of all women, no risk whatsoever, about one to one and a half percent will carry some of these small micro deletions or duplications, also called copy number variants.

So individuals began when they did diagnostic procedures like amnio or CVS, began to look for these. Now the last change which occurred started about 10 years ago, but really maybe six or seven was noninvasive prenatal testing. Although amnios and CVS dramatically reduced their risk, as they said it was one percent when we started doing it without ultrasound. The risk of losing a pregnancy because you have one of these procedures is somewhere between one in 700 to one in a thousand, almost a tenfold in improvement, but still it had a risk. So patients were faced with the decision of whether or not they were willing to take that risk to get the genetic information. Along came noninvasive prenatal testing, in which we could draw a sample of blood from the mother. We realized that about 10 to 20 percent of the DNA in that sample from the mother was fetal DNA, and then using sequencing techniques, which I believe we'll talk a little more about in the future.

Using sequencing techniques, we could identify which pregnancies had Down syndrome without having to do a procedure. Again, just as with every new test, we had to figure out how it compared to what was being done previously. And with noninvasive prenatal screening, we were able to go from around the 80 to 85 percent detection rate of the abnormal number of chromosomes to 99 percent. So we can identify 97 to 99 percent of Down syndrome pregnancies. More importantly as I said, we started by offering testing to 15 percent of women because they were maternal age. As screening got better, that number went down to three percent, but still that's a large number of 'false positives.'

So with noninvasive DNA testing from the mom's circulation, one in 1000 to one in 10,000 false positives occur. So we dramatically decrease the number of patients that would need diagnostic testing to confirm with the blood shows.

Paul: Interesting. And one of the things he said I'd like to pick up on is you talked about noninvasive prenatal testing, but you referred to it as screening. So I'm wondering if you can talk a little bit about the difference between screening versus diagnostic testing.

Dr. Wapner: Yeah, that's a really important point. A screening test is a test that is intended to be used on the whole population not to diagnose a problem, but to identify the people at the highest risk for the problem. And we screened with the age, we screened with the chemicals, we screened with looking at the DNA. But it's not a diagnostic test. There are a small number of false positives in which the screening test says you're at high risk and the pregnancy is completely normal, and false negatives in which the test says everything is okay, but it's really not. So, a screening test identifies those individuals whose risk is high enough that they then should undergo the diagnostic tests. In general, diagnostic tests have slightly more risk, are slightly more expensive, a little more complex. So you can't offer diagnostic testing to all 4 million births. So the screening test helps us identify those pregnancies at risk, and those that come out that have the highest risk than have the diagnostic test in order to confirm that indeed the fetus is affected with whatever the screening test said.

Paul: So, for the screening tests, the other thing I'd like to pick up on is you mentioned that the population of patients that are candidates for noninvasive prenatal screening is higher than at risk patients. So in terms of the kinds of patients you see, what types of patients do you council to get this kind of screening? Is it everyone or is it still only patients who are a certain age?

Dr. Wapner: Every woman, I mean the American College of OB/GYN and actually now the standard of care is that every woman is offered the opportunity to have her pregnancy screened for the common chromosomal abnormalities. There's still a bit of a transition. Some people are still having the chemical and ultrasound evaluations. Some people have moved on and begun to get noninvasive DNA testing, but every woman should be offered that. And actually to be honest, it's close to beneath the standard of care not to offer it. Now, that doesn't mean that every woman should get it. There are a lot of people that just don't want that information for tons of different reasons, but they need to know about its availability and about the possibility. The American College of OB/GYN still says not only that, but every woman should be offered a diagnostic test, either a CVS or amniocentesis. And the woman needs to be counseled or informed that there's a difference between screening and diagnostic. But if a woman says, "I want more information, I want to find those microdeletions and those microduplications. I don't want to take any risk whatsoever," then they have the option of having the diagnostic test performed. So really every pregnant woman needs to be informed of this. Not everybody has it, probably about half make that decision. Probably a little bit over half now. But yeah it involves every pregnant women.

Paul: Wow. So if that's the case, and you just mentioned something about offering some counseling and some guidance to help women make that decision. If there's 4 million

pregnancies, that's a lot of counseling. So how do you handle that? How do you get the message across to your patients and describe these different technologies?

Dr. Wapner: You hit the nail on the head. And I stumbled over the word when I said counseling and then I switched it to education. Genetic counseling is a really in depth discussion of genetic issues that are very pertinent to that individual patient. There's about 3000 genetic counselors available. Many of them don't do reproductive counseling.

Paul: And, that's not a big number overall anyway.

Dr. Wapner: No. So for 4 million births you couldn't have everybody be genetic counseling, but they don't need counseling. What many many women need is education. What are my risks and what are the available technologies? And you can do education in a number of different ways. One way is every doctor who's seeing his patient in the first trimester informs her and has this discussion. The average obstetrician sees an OB patient for somewhere around 15 minutes per visit and particularly at that first visit, they have to talk about what to eat during the pregnancy, what blood tests need to be done, what about the risk of preterm birth, how do you get in touch with the office, how do you pay your bills? It's really getting to the part where it is impossible to have that much time to talk to the patients about this.

So we really need and have developed other techniques, and there are now websites that are dedicated to educating women about their prenatal screening options, and their prenatal risk. And the Perinatal Quality Foundation nicknamed PQF has developed a process called the GEM, which is the Genetic Education Module which is being made available to practicing obstetricians so that they can say to their patient before you come see me or even after the first visit, go to this website. It has a very short 20 or 30 minute overview of the risks. But then the woman has the option of going into more detail if she wants that. She then can type in any question so that when she gets to her physician's office, she can have those questions answered. Some patients won't be sure what they wanna do. They need to see the genetic counselor. So, we've not only streamlined the most important patients to test. We've also streamlined the patients that need the real long discussion. Because for some patients this is a very difficult decision.

Paul: Right. I mentioned there are some patients that are at risk. And for those patients, I guess it's a much easier decision on the kinds of testing. But for someone who's an average risk woman, what kinds of information do you think they are looking for when they're trying to decide what kinds of testing or screening that they should do?

Dr. Wapner: I'm going to narrow or discussion for a session because there are other things which I'll get to, but we're talking specifically now about the woman's risk for having a child with a chromosome problem such as trisomy 21, 18 or 13. So they really need to understand their risk of having a child. They need to understand there are other genetic conditions that can be diagnosed. They need to understand that there's availability of diagnostic testing, and the availability of screening what the risks and the benefits of each are. In other words, the decision they have to make between a diagnostic test with more information, which many people choose that's what they want, versus noninvasive

testing in which you don't have to have a procedure. In short, that's really what a patient has to understand from screening for chromosome abnormalities. In addition to what these chromosome abnormalities are, and we're having a discussion because we're both familiar with medicine and genetics. And if we say you have a risk for Down syndrome, everybody knows that means an extra number 21 chromosome, and that's trisomy 21. But it doesn't do to say to a woman that blood test you're going to have is looking for Down syndrome without explaining to her what that means.

Because many people relate the term Down syndrome to any child that has any genetic disorder, and there's no reason they shouldn't. So like if I said to you go get me a coke, you would bring me a brown soda. So I think that patients in making that decision have to understand that there are many more genetic disorders than just Down syndrome. For instance, a 40 year old woman because the risk increases with her age, Down syndrome is really what she needs to worry about. But for a 25 year old woman, her risk of having one of those smaller copy number variants is two or three times more frequent than Down syndrome. So they very well need to understand what their risks are, and these websites have that kind of information. Why I wanted to narrow the discussion because another risk that we've now begun to screen for are risks of having what are called Mendelian disorders.

They're not caused by any number of chromosomes, but they're caused by a specific change, not only in a gene, in a tiny little part of the gene. And each of us carry somewhere around 12 genes that are abnormal. Well most of these aren't important because they are only problematic if their partner has a problem in that same gene. So individuals that have this, they're inherited in what's called an autosomal recessive way in which both parents need to be carrying one of these errors in a gene. And then only when couples both have the same issue, then they have a chance of having an affected child.

And about 10 to 15 percent of all admissions to children's hospitals are caused by genetic diseases. So the ability to identify at risk pregnancy, and I don't want to make it sound like if both parents are carriers that their only option would be if they have an affected child to terminate the pregnancy. Because knowing some of these disorders before birth allows the neonatologist to treat them. We can sometimes modify the course in utero. So we're now showing that identifying a genetic disease in a child is really important, particularly if a child in intensive care nursery who's having severe problems like shock or coma, etc. Some people were actually going to sequence those children as the first test.

Paul: It's sort of analogous to the research situation. Reason a lot of research scientists like these large data genomic approaches is it's unbiased, so it gives them appreciation of genes that they didn't anticipate before. And it sounds like in the clinical setting it's sort of similar. This unbiased approach is giving you the appreciation of the clinical manifestations of for example microdeletions that you probably wouldn't have known if you're specifically looking only for a trisomy.

Dr. Wapner: Unquestionably correct that in prenatal diagnosis, and as you had to bring up I've been doing this for a long time.

Paul: 30 years.

Dr. Wapner: In prenatal diagnosis, it used to be identify the phenotype, ask the question, “What are the diseases that can cause this phenotype?” And, then test for those specific things that you could test for. And, when I say identify the phenotype I'm talking about by ultrasound. It's changed completely, because we now have the ability through screening or for other techniques, identify the genotype first and then we can use the ultrasound to evaluate the evolution of the phenotype. It's a total 180-degree switch. And I think if I talk to you in five years and you say to me the same question, what has changed, the change will be we've gone from phenotype, see if it's a genetic disease, to identify that it's a genetic disease and then see what the manifestations of the phenotype are, be total switched.

Paul: Yeah, that's completely opposite. So one thing that you said that I'd like to talk a little bit about is that you said with noninvasive prenatal screening, there's a 99 percent success rate at determining trisomy 21 for example.

Dr. Wapner: That's correct.

Paul: So there's this concept of predictive value. So that 99 percent effective screening, how does that translate into whether or not a detection of trisomy 21 or detection on a noninvasive prenatal screening is actually going to lead to some clinical outcome that you need to treat?

Dr. Wapner: Yes, and I think your question is quite important. People sometimes get confused. The 99 percent is sensitivity. That means of all pregnancies that have trisomy 21, this screening test will identify 99 out of every hundred. That doesn't mean that a woman who has a positive test has a 99 percent chance her fetus has it, because there are false positives and false negatives.

So the positive predictive value takes into consideration the woman's risk before she had the test. So if a woman's 40 and the blood tests were positive for Down syndrome, it's highly likely that's Down syndrome because her risk of having it. But a 20 year old woman who has a same exact positive test may have a one in 2000 risk. So a positive test is more likely to be a false positive than it is to really be affected. So trisomy 21, because it's 99 percent, isn't the perfect example. But for instance, trisomy 18 in which we only identify 97 or 98 percent and have a few more false positives. As I said, a 37 year old woman with a positive test is like 99 percent that it's trisomy 18, but in a younger woman, she's got more than a 50 percent chance that that test is actually a false positive test.

Paul: Wow. That's quite high.

Dr. Wapner: And with sex chromosome abnormalities an extra X chromosome or a missing X chromosome, the sensitivity and the false positive rate is lower. So it really has a relatively less positive predictive value. So sensitivity and specificity is what the public health person wants to know. I have a new test. How many cases of this is it going to

identify? But for Mrs. Smith or Mrs. Jones, she wants to know if she has a positive test. What's the positive predictive value? What's my risk? And I think it's really important that people know the difference because a 25 year old woman who's more likely not to have a problem than have a problem should never consider making an irreversible decision just on a screening test. She absolutely has, well everybody that has a positive screening test has to have a diagnostic tests, but the risks of a positive test are very different depending on the age of the patient.

Paul: Interesting. Before I get to the last question, I just wanted to come back to something that you mentioned, which is the type of genetic perturbations that you're looking for. So we talked about some of these aneuploidies, so noninvasive prenatal screening. What are the types of genetic abnormalities that are most effective to be screened with that test?

Dr. Wapner: Yeah. We're again going back to chromosomal abnormalities. Whole chromosome aneuploidies, abnormal number of chromosomes for 13, 18 and 21 are really the ones that are most appropriate, what we call the common aneuploidies. Why? For two reasons. The testing has been worked out well, but they're the most common things, particularly as a woman gets older, so they would have really high positive predictive values. Although as I said, it's a little less good for 18 and even a little less good for 13 and less good for sex chromosome abnormalities. Now you get to the microdeletions, and the microdeletions as I said occur in about one to one and a half percent of all pregnancies. However, there's lots more microdeletions and duplications.

Paul: Right, I read that in aggregate they're more common than trisomy 21.

Dr. Wapner: But each one is less, and the way one does noninvasive screening, and I will say a word about that, you actually have to target what you're screening for. So when you're screening for trisomy 21, you count the pieces of DNA that are floating around in the mother's circulation from that chromosome. Since microdeletions and duplications can come from many chromosomes, it's just not feasible right now to do that. Now you could, if you counted thousands and hundreds of thousands of pieces, identify tiny little missing pieces, but you really can't do that because you just can't sequence that. It would just cost an infinite amount of money. When you're looking for a whole chromosome, you've got all the pieces from a whole chromosome, not just the piece of the chromosome. So that's why we can do that. So every lab right now offers some microdeletions on their panel, but only a minuscule number of all of those that exist.

Paul: Interesting. Okay. So final question before I let you go back to your day job. What is exciting you about-

Dr. Wapner: This is my day job.

Paul: What is exciting you about the future of genetic testing, and in particular, what do you think the next few years is going to hold in terms of development of noninvasive prenatal screening since this is becoming so important?

Dr. Wapner: The ability to screen for different genetic diseases continues to improve and we've talked a lot about that. We've gone from identifying whole chromosomes noninvasively, we're moving as sequencing improves to little pieces of chromosomes. The last horizon is to noninvasively be able to sequence a fetus, to look at every base pair. You can do that. You can absolutely do that today. The feasibility, biologic plausibility to do that has been proven. One can not do that as a clinical process. Whether one would want to do that as a clinical process is also a question because it's just too labor intensive. The first time they sequence the fetus it took six months to a year to do that. You can't wait for results.

But, that technology will come because right now when we look at noninvasive testing, we have a mixture of fetal DNA, 10 percent, and 90 percent maternal DNA. So, we have to go through each piece not knowing whether it's the piece from the fetus or whether it's a piece from the mom, and then do kind of mathematical calculations to ask is there too much coming from fetal chromosome 21? If we could find a marker that allows us to differentiate the pieces that come from the fetus, from the pieces that come from the mom, the game changes completely because then we could isolate the fetal DNA and there's two approaches to doing that. Separation where the fetal pieces actually are slightly smaller than the maternal pieces, so you could do it on size, but most of this which we didn't talk at all about this, but the bioinformatics, how the computer has allowed us to do things, there are some thoughts that there are some bioinformatics, subtle DNA changes that can help us separate that. And that's probably where we would go. And once we can do that, we would be able to sequence the fetus.

Now, I'm not suggesting that that's the right thing to do. The argument is how much should you know about the DNA of a fetus? But one has to take into consideration that one of the main causes of birth defects are fetal structural anomalies, congenital birth defects. It’s three percent. The general risk of down syndrome in the general population is one in 800. The general risk of a recessive disease that we could pick up with pan ethnic screening is one in 500. Microdeletions are maybe one in 90. Birth defects occur in three percent. And the vast majority of these are caused by single base pair mutations, and they’re *de novo* mutations. So you can't screen for them. So they occur only in that fetus, not necessarily in the parents.

So noninvasively, we can eliminate already by carrier screening the majority of the recessive diseases. We can get some of the major chromosome abnormalities, but the 900 pound gorilla is the genetic etiology of some of these birth defects. So where that will start is we're not going to look for everything. We're going to look these are the most common genes that are associated with the most common birth defects, and we can begin to start looking for those. And actually, there's a company already that has a test that looks for 30 of the most common genetic mutations in a fetus. And they're talking about screening the whole population. The last piece of what could happen, we've talked about cell-free DNA, and I've mentioned the difficulty separating it from the maternal DNA, which makes it a little more difficult.

There are people that continue to work on retrieving fetal placental cells, trophoblast cells from the maternal circulation. They're getting good, but not perfect. If you could get three fetal cells, it's only fetal DNA. You could amplify the DNA, you could analyze

the DNA, and you could get the answer to all of these if we can get cells. Will we do it? When I say 50 years ago, for 30 years I've been involved in one way or another trying to find the cells. They're tough. In a total tube of blood you would have drawn, there might be 10 to 30 of these cells. So they're really infrequent. But the microdissection, microisolation techniques are getting better and better. And that conceivably could happen. So that's what we'll be talking about, diagnosing single base pair variations.

Paul: Awesome. Dr Wapner thank you for joining us. I think our listeners have a very good sense of what prenatal genetic testing is and how it's used clinically. Thanks for joining us on the Illumina Genomics Podcast.

Dr. Wapner: It's my pleasure to do that.

Paul: According to ACOG, every woman should be offered the opportunity to have her pregnancy screened for the common chromosomal abnormalities. The advent of NGS-based NIPT has expanded the options for prenatal chromosomal screening for women in pregnancy, and NIPT is 99% sensitive for detecting trisomy 21. It's important to understand that NIPT false-positive results are more frequent among low-risk women than those at high risk, so a positive screening test needs to be confirmed by a diagnostic test. If you're interested in learning more about GEM for education of patients and physicians about prenatal screening and testing options, visit gem.perinatalquality.org. If you liked today's show, please subscribe on Apple Podcasts or Google Podcasts. Join me next time, when I'll be talking with Dr. Sam Sternberg, Assistant Professor of Biochemistry and Molecular Biophysics at Columbia University. We'll be discussing CRISPR-Cas biology and genome engineering - here on the Illumina Genomics Podcast.