Validating a genetic biomarker tied to miscarriages
AMA
MAKING THE ROUNDS
Validating a genetic biomarker tied to miscarriages, 2022 AMA Research Challenge

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In this episode of Making the Rounds, Leelabati Biswas, an MD/PhD candidate at Rutgers Robert Wood Johnson Medical School, shares about her research: "Decoding Pregnancy Loss, Validating a Novel Genetic Biomarker of Poor Egg Quality."

The AMA Research Challenge is the largest national, multi-specialty research event for medical students, residents and fellows, and international medical graduates to showcase and present research.

Speakers

- **Leelabati Biswas**, MD/PhD candidate, Rutgers Robert Wood Johnson Medical School
- **Brendan Murphy**, senior news writer, American Medical Association

Host

- **Marielisa Cabrera-Sánchez**, 2021 AMA Research Challenge winner

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Transcript

URL: https://www.ama-assn.org/about/research/validating-genetic-biomarker-tied-miscarriages

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Murphy: Hello and welcome to Making the Rounds by the American Medical Association. I'm Brendan Murphy, senior news writer at the AMA. Today, we are joined by Leela Biswas. Leela is one of the five finalists for the AMA Research Challenge. Hi, Leela. How are you today?

Biswas: Good. I'm doing well. Thanks so much for having me.

Murphy: We are very excited to have you. Leela is pursuing an MD/PhD at Rutgers Robert Wood Johnson Medical School. Her submission poster for the Research Challenge is entitled, “Decoding Pregnancy Loss, Validating a Novel Genetic Biomarker of Poor Egg Quality.” Let's get into your research, Leela. Can you tell us a little bit about this topic, why it appealed to you and how you got involved in the AMA Research Challenge?

Biswas: Sure. Happy to discuss. My research really deals with the disease of infertility. It's a topic that's not widely discussed, I think, because the experience of miscarriage and of infertility is really painful for individuals and for couples. As some background, roughly one out of every 10 female-identifying individuals in the United States are diagnosed with infertility, so it's a lot more prevalent than people realize and it's incredibly devastating for individuals who want to get pregnant but can't. About 25% of pregnancies end in miscarriage, so really strikingly common, but the leading genetic cause of miscarriage is something in the embryo called aneuploidy.

Aneuploidy is a chromosomal abnormality in the cell. Basically, the cells have the wrong number of chromosomes, an incorrect number of chromosomes. When you have an aneuploidy egg and it gets fertilized by a sperm, it nearly always leads to miscarriage via an aneuploid embryo, but the only biomarker that we have for this is maternal age. It's a J-shaped curve, which means that, on average, people, as they get older, tend to develop more aneuploid eggs and that skyrockets around 30 to 35 and the chances of a successful pregnancy go down with that.

Maternal age is actually a really effective predictor for egg aneuploidy but it doesn't work for everyone. They're individuals who are at an advanced maternal age and have very few aneuploid eggs, and people who are very young and have a lot of aneuploid eggs and unsuccessful pregnancies. If age isn't predicting aneuploidy for these individuals, there has to be something else at play, some other factor and so we hypothesized that there's a genetic contribution as well, that some people have more egg aneuploidy than you'd expect for their age because of their genetics. If we could identify those genetic factors, then you can imagine what we might be able to do with that, is to identify a predicted...
genetic biomarker so that we could identify them, not the invasive testing or just certify having them get pregnant and then lose the pregnancy over and over again but by a simple blood test well in advance of actually trying to conceive a child.

The thought is that a physician in the clinic, before somebody has decided to get pregnant, sits down with somebody, sits down with the patient and does a blood draw, runs a genetic test for biomarkers, combines that with maternal age, and then they can together design a cure plan and a pregnancy plan that works for them. Maybe that is starting a family earlier if there's somebody who is going to have a lot of aneuploid eggs at an earlier age than you'd expect or it might be egg donation. It might be IVF. It might be adoption. It's whatever makes sense both clinically and personally for the patient and the physician, if that makes sense.

What we did here was to take a cohort of patients, pull out those individuals who had statistically extreme rates of egg aneuploidy relative to maternal age and then use whole exome sequencing to identify genetic variants that were enriched in those high aneuploidy people. And then we took those variants, and we asked, "Well, do those variants really cause aneuploidy in those patients or is that just a coincidence that some high aneuploidy patients have these genetic variants?" We tested those variants in actual mouse eggs. We over-expressed the variants in mouse eggs using microinjection and asked if the variants in question could disrupt myosis, basically mess up how the egg divides and segregates its chromosomes, causing aneuploidy.

In fact, we found one that did and then the question was, well, that's in a dish, those are cells in a dish and you're overexpressing them, so they're not at physiological levels. What happens if it's in a whole organism? What happens if it's at a physiological level? Does that variant still cause egg aneuploidy? We generated a novel knock-in mouse with the help of the Rutgers genome editing core using CRISPR, and we took that patient variant in question and knocked it into the mouse. The mouse had the same patient variant and we asked, "Does this mouse have increased aneuploid eggs?" It turned out it did. Compared to its age-matched peers that had a wild-type copy of the gene, the mice with the mutation in question, that we found in patients, had an increased rate of aneuploidy.

It was really exciting and our hope is that this would lay groundwork for then developing those biomarkers that we can use to predictably identify patients well in advance of attempting to conceive.

And this appealed to me because I previously had volunteered for two years with children with developmental disorders, as well as did research and cancer biology. And so, as I went through my education, I learned more and more about the genetic basis of developmental disorders, as well as how genetics could be applied to many fields of medicine to understand the origins of disease. I was obviously very excited to learn about this project where I get to learn about the unraveling of genetic data to really understand how disease and, in this case, egg aneuploidy and miscarriage can arise during development.
I was also able to learn how to use broadly applicable scientific tools like mouse models and classical genetic approaches, high-resolution confocal microscopy—you'll have to check out my poster to see those pictures—and dig deeply into cell biology and genomic stability which are broadly applicable biological concepts. I'll anticipate that I'll be able to use those tools as a physician-scientist to tackle the most pressing diseases when I'm a fellow or an attending.

Murphy: Well, that certainly is research and work that could really affect the lives of many, many patients and certainly seems translatable. What were some of the challenges you encountered in doing this research, Leela?

Biswas: Sure. The biggest challenge, I think, this is a really multi-pronged project with a lot of moving parts, so we have computational collaborators, as I mentioned, that we pull data from bio-banked genetic sequencing data from patients. We have computational collaborators who are able to analyze those data and extract the most enriched variants. We collaborate with them closely and then work through the biological end, and so we have to have a lot of back-and-forth between the two groups. We need to be able to closely collaborate and also be able to comment on each other's work because they understand the computation, we understand the biology and the clinical aspects of it. And so there's a real back-and-forth there in terms of the coordination.

In addition, I'm using a mouse model and a novel mouse model. It takes a really long time to get a new mouse model up and running. Mice have a gestation time of about 20 days, and then, they take another six weeks to be sexually mature. It's a long time between having the mouse that you need born and being able to actually do the experiment that you want to do, and then you need multiple replicates. Sometimes experiments don't go as planned, and so that really slows down the process and makes it very labor intensive but extremely rewarding because a mammalian model is essential, I think, in this, in the setting of this experiment.

And then also all of the challenges of research. So often, experiments don't go as planned, you have to repeat things, and so, it's a lot of that troubleshooting and reworking and learning so many new techniques. I had never worked with a mouse before joining this lab. Learning high-resolution confocal microscopy with a million-dollar microscope, that was really tough and scary. It takes a lot of failure and being able to pick yourself back up and learn and ask questions and go to others for help and do all the research to figure out how to make things work. It's that difficult process of science, of failing and then just trying again in smarter, hopefully, ways to eventually succeed.

Murphy: What advice would you offer to medical students who are conducting research on a project like this?

Biswas: So first, right off the bat, you need to find a project that you're really excited about. If you don't have something that you're super thrilled to be doing, it's going to be really difficult to do it as you experience those failures and challenges in science. I'm thrilled to be doing my project and I'm really...
hopeful about how we can positively impact the lives of patients, and that really drives me forward every day. Find something you are excited and passionate, deeply passionate about.

Second, be prepared for challenges. As I mentioned before, research has tons of failures and hiccups and troubleshooting before your success, during your becoming successful and then after you've been successful. You will just continue to find failure, and so, you have to be able to get knocked down, fail, pick yourself up, learn from it and keep going. Be prepared for that and then also be ready to learn all the time.

As I mentioned, I had learned a lot of new techniques in doing this project from the microscopy to the mouse genetics, to Oocyte biology, to 4D image processing software. There's so much to learn in a new and exciting project. The more exciting your research is, probably the more you'll be learning as you do it. I think that's the key to a lot of science and probably a lot of clinical medicine, although I'm still a student.

Every field of medicine is just accelerating so rapidly. There are always new techniques. There's always more data to be found. If you are static, you fall behind. You just have to keep being like a sponge and learning more and more and more and accelerating. You can't be afraid of that. You're not going to get everything but you just have to keep learning and being excited about your science and about your medicine and remember why you're doing it, which is for the patients.

Murphy: Can you talk about how these findings could maybe contribute to a change in the way some patients go about having a child?

Biswas: Sure. What we did was identify a genetic variant that increases the rate of aneuploidy in mice and is enriched in patients with high rates of egg aneuploidy. Our hope is that this will lay the groundwork for this variant to become, potentially, a genetic biomarker. The idea is that, prospectively, in the clinic, before people are ready to have a child, before they're conceiving, that they would receive a genetic test for this and other biomarkers that are well validated, well studied in large cohorts long-term, which we haven't done yet. That's an important next step. They would receive genetic testing and, that, the results of those genetic tests, could be combined with maternal age data, because that's still a critical important, fundamental factor in causing egg aneuploidy. And then the patient could talk to their physician as well as their genetic counselor about the results of those tests to determine when is a good time and what is a good strategy for them to achieve a successful pregnancy.

For example, if there's a patient who has a lot of variants that predispose them or one variant that predisposes them to having a high rate of aneuploidy at a young age. So, say, for example, maybe they'll have a lot of aneuploidy at the age of 28, which is usually an optimal time to have a pregnancy based on what we know about maternal age outside of variants, so if they have a variant that causes them to have that high aneuploidy, maybe they want to freeze their eggs at an earlier age before their aneuploidy increases. Maybe they want to consider egg donation. All of those interventions would
have to be tested empirically to determine the risk and benefit long-term in patients.

Right now, we’re just laying that groundwork but the idea is that this would be integrated, so genetics would be integrated with maternal age in the clinic by a physician, with the patient involved, to design a really personalized care plan. It’s strategic pregnancy planning to avoid some of that heartbreak, to avoid the pain of miscarriage that so many people are facing.

Murphy: We talked about the big picture plan, how this applies there. What are the next steps in this work?

Biswas: Sure. There’s short term and there’s long term. In the short term, in terms of what I’m doing in my next steps in my thesis work, I’ll be using this novel knock-in mouse line as well as a couple of other mouse lines that we have in the lab to explore the mechanisms behind the mutation that we identified, KIF18AMDM, how that causes aneuploidy in eggs. That sounds like a really basic, fundamental science question which is always incredibly valuable to explore the basic side but it’s also important, I think, clinically and translationally. When you have a mutation in a gene that causes an amino acid substitution or change in protein, learning more about that specific region of the gene and protein can help you understand how other mutations that people have can affect that same gene or protein and then, long term, lead to potentially a similar phenotype.

It doesn’t mean that necessarily, if somebody has a mutation at spot 25 that another person at spot 26 will have the same disease as person A but it does mean that, perhaps, if you find another patient with the same phenotype who has a mutation nearby that that might be a good candidate to study, that there’s established evidence that might lead you down that road. Really exploring those mechanisms and understanding how the protein works and how a given mutation can disrupt the function and lead to a specific phenotype is really important and can also lead to the development of targeted therapies down the road.

And so that’s what we’re going to be exploring next, really digging into those mechanisms on how KIF18AMDM causes aneuploidy in mouse eggs, and then, long term, the lab wants to identify more mutations that cause aneuploidy and validate them in knock-in mouse models. That work is ongoing in multiple genes using the same pipeline, so basically identifying mutations that are enriched in high aneuploidy individuals and validating those in mouse models, and then, downstream, the really critical work in translating this is going to be validating this in a large human cohort. Our hope is to really identify those causal variants in our population with our biobank data, biologically validate those in mice and then clinically validate in large human cohorts and, hopefully, translate those work.

Murphy: It certainly sounds like a process but it does sound like you’re on the right and exciting track.

Biswas: Thanks.
Murphy: You yourself are in the process of forging a career toward becoming a physician. How does this work impact your career trajectory or does it?

Biswa: Yeah. Yeah. Definitely. I mean, the big thing is the intensive research training combined with my clinical training is incredibly valuable. It has armed me and equipped me with not only the scientific thinking and the approach to rigor and reproducibility that are going to be critical as a physician-scientist but also a number of useful tools such as classical mouse genetics, such as high-resolution microscopy, imaging, being able to really see inside the cell. We can do that. It's amazing. I'm going to be able to use those tools long-term as a physician-scientist in the future, and then, more in terms of the topic of my research.

My research really focuses on precision genetics, and so really digging into large amounts of sequencing data from individuals to identify genetic variants that we can correlate with phenotypes and disease states in the hopes of identifying therapeutics, diagnostics and prognostics. That's something that has really shaped where I want to go in my career. We have so much omics technology now from the genome and exome to the protium and beyond that transcriptome, and on and on and on. There's so much data that we can pull from each of us, from each patient and each of ourselves to identify, what makes me, me and what makes you, also makes me have one disease and you potentially have another disease and how that shapes how our treatments might be different, and what might be optimal for me might be different than what's optimal for you to achieve a healthy and happy life.

And so really driving towards precision medicine. That has really been...this project has really shaped my desire to become somebody who is a physician-scientist that specializes in precision medicine. That's where all the fields of medicine are going, I think, in some way as we gain more and more data. I'm excited to be on the forefront of that and be part of that army of precision medicine physicians really optimizing care plans for patients.

Murphy: That certainly is the future. What else should our listeners know about your journey in medicine?

Biswa: Sure. My journey has been the weaving together of many valuable experiences. I spent two years volunteering with children with developmental disabilities in high school, and then I did intensive research in undergrad at Thomas Jefferson University's Kimmel Cancer Center in college, and then my medical school studies have really integrated for me all of these myriad of experiences in that biological and clinical context to help me identify my passions. Along the way, I've also had really great mentorship and collaborations. And so, together, all of this has led to exciting MD/PhD research and for which I'm fortunate to have received NIH funding in the form of an F30 predoctoral fellowship. I'm extremely lucky to be doing the research I'm doing at Rutgers and also to have received funding to continue my project.
Murphy: So, as you know and I think our listeners will know if they've listened to other episodes of this podcast, the Research Challenge winner is awarded $10,000 from our sponsor, Laurel Road. What would you do if you won that money?

Biswas: Yeah. Well, there are a lot of expenses involved in an eight-year graduate MD/PhD program. It's twice as long as the average medical school, so I would probably invest in supporting some of those educational expenses so that I can become a physician-scientist and then meaningly give back to my patients and my community. I'm very lucky to be participating in the Research Challenge and to be advancing my academic career so that I can do meaningful work for patients.

Murphy: It does seem to have a lot of translational value and really could affect the lives of patients going forward.

Biswas: Thank you. I appreciate it, Brendan.

Murphy: Absolutely. Leela, this is important work being done here. Thank you so much for joining us and sharing it with us.

Biswas: Thank you so much for having me. I appreciate it. Have a good day.

Murphy: A quick reminder for our listeners, you can see Leela’s research poster, as well as those of the other finalists in the Research Challenge, at that Research Challenge website, which is ama-assn.org/research22. Of course, be sure to tune in to the finals of the AMA Research Challenge on December 7, to see all five finalists present their work to a panel of expert judges for the chance to win that grand prize. This has been Making the Rounds, a podcast by the American Medical Association. I'm AMA senior news writer Brendan Murphy. Thank you for listening.

Cabrera-Sánchez: Join us on December 7 at 7 p.m. Central time, to see all five finalists present their research to an elite panel of judges. The overall winner will receive a $10,000 grand prize—sponsored by Laurel Road. For full details, visit ama-assn.org/research22.

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