Nurturing the Field of

PRETERM BIRTH RESEARCH

BURROUGHS WELLCOME FUND

By Sandra J. Ackerman

A successful human pregnancy, the assembly of an entire person within a closely confined space, is a feat that depends on precise timing. Each layer of cells and every incremental stage of development has its appropriate point in the sequence.

Nevertheless, as with any intricately coordinated process, there can occasionally be a hitch in the timing, and in the case of pregnancy this means risk. Babies born with less than a full term of gestation may be vulnerable to a range of health problems, from the mild and short-lived to severe and lasting. Such is the situation of some 380,000 preterm babies born in 2016 in the United States—a prevalence of almost 10 percent, one of the highest rates among industrialized nations.

There appears to be no single explanation for this high rate in our country, or for preterm birth in general. It's not surprising that this should be the case, says Jerome Strauss, professor of obstetrics and gynecology at Virginia Commonwealth University. Since 2009, when the Burroughs Wellcome Fund launched a grants program called the Preterm Birth Initiative (PTBI), Strauss has served on the program's advisory committee. In his words, "We don't really understand what determines the length of normal gestation, let alone the factors that interfere with the normal course."

A colleague on the Advisory Committee and a fellow professor of obstetrics and gynecology, **D. Michael Nelson** of the Washington University School of Medicine, points out that pregnancy presents a unique biological challenge, similar to the body being coaxed to accept a transplant (but with a different timeframe, of course). Researchers today generally agree that preterm birth—with anything less than 37 weeks of gestation—is a multifactorial problem. Many factors may play a role, including physiological stress, cervical disorders, and immunological processes that are either inherent or responses to infection. Likewise, many different approaches today offer promise for understanding and reducing preterm birth: microbiology, immunology, genomics, and more.

At the Burroughs Wellcome Fund, the idea of establishing the PTBI originated with pediatrician-virologist George Miller and coincided with a report from the Institute of Medicine report on the same topic. Says Strauss, "I can't take credit for the idea, but I immediately and enthusiastically endorsed it, because it was a major step forward." The initiative came at a time when the stock market was very volatile, Strauss recalls, adding, "I have to give credit to John Burris and the staff, who were heavily involved in getting this underway, for taking this step at a time when resources were uncertain."

Jeffrey Whitsett, co-director of the perinatal clinic at University of Cincinnati Children's Hospital and the University of Cincinnati Department of Pediatrics, serves on the PTBI advisory committee. He points out that prematurity is an under-appreciated cause of infant mortality and injury. With the underlying causes often unclear, preterm birth remains an unsolved medical and social problem. In fact, says Whitsett, "It's one of the leading causes of death among newborns worldwide." A preterm pregnancy may be difficult to detect, but the need to encourage and support research in this area is all too apparent.

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Louis Muglia, the other co-director of the Cincinnati perinatal clinic, also heads up the Center for the Prevention of Preterm Birth and the Division of Human Genetics at the University of Cincinnati. As chair of the PTBI advisory committee, he considers the aim of this grants program to be "looking for exciting new ideas and new insights. We want to see proposals from people already working in the field, but also from people working with cutting-edge technology, using approaches that haven't yet been applied to this problem."

Strauss adds, "It's encouraging to see newer investigators, in particular, engaged in genomics, immunology, infectious diseases, and the like. There are large-data, bioinformatics approaches, both animal models and translational-research approaches." With regard to the criteria for applicants, **Susan Fisher**, also on the advisory committee and a professor of obstetrics, gynecology, and reproductive services at the University of California-San Francisco, says, "In terms of experience or academic rank, neither end of the spectrum is penalized. . . . Grants go to a broad range of people, from early-career assistant professors to established professors who are already running their own lab." In a nutshell, says Muglia, "We're really just looking for the best ideas."

A GENETIC PERSPECTIVE

With a variety of risk factors for preterm birth already known, and possibly more that have not been identified yet, attempts to characterize the unknown factors can leave researchers trapped in a paradox. Geneticist **Ge Zhang**, of Cincinnati Children's Hospital Medical Center, puts it this way: "Maybe some researchers say we should collect more homogeneous samples, but we can't collect more homogeneous samples until we know what the risk factors are." An awardee of the PTBI class of 2017, Zhang sees a need for prospective studies with long follow-up, perhaps as much as 20 years; unfortunately, in the United States this type of study is relatively rare, because the patchwork nature of our healthcare system makes it difficult to collect the kinds of consistent, long-term records that would be required.

But even within the records that are available, the genetic approach to studying preterm birth comes up against another challenge: defining the object of inquiry. Zheng explains that in standard genetic studies, the goal is usually to link one genotype to one phenotype. But in the study of preterm birth, it's not clear which phenotype to focus on: that of the fetus or of the mother? "We can definitely study the genome of the mother or of the baby, but pregnancy involves an entity that is neither of these," he says. Zhang see this as a key challenge, but also a key opportunity.

"Our approach," he goes on," is to regard mother-and-fetus as a single unit. For example, preeclampsia—one of the major causes of death related to pregnancy—is usually regarded as a problem that originates in the mother, but our genetic studies suggest that at least part of the risk for this comes from the fetus."

Biochemical signals of all kinds flow not in a one-way path from the mother to the developing fetus, but in a continuous loop between the two. "If we can collect genomic data from mother-fetus pairs to look at the cross-talk, we can find out something about how the fetus can influence the mother," he says. As a further goal, Zheng hopes to uncover clues about possible associations between preterm birth and the development of certain chronic diseases later in life, such as cardiovascular disease and diabetes.

IMMUNOLOGICAL PERSPECTIVES

Like Zhang, **Tippi MacKenzie** received a PTBI grant in 2017; unlike Zhang, however, MacKenzie approaches preterm birth research through the prism of the immune system. "The unique world of the fetal-placental unit is arguably the most important immune environment we have for the survival of the species. And it's such a complex system that it's probably impossible for any one person to study it all," she says. In her precision-medicine lab at the University of California-San Francisco, "The novelty of our work is that we're focusing on the fetal immune system."

Normally the fetus doesn't have a strongly developed immune system, nor does it need one—the mother's immune responses protect the fetus as well. But in analyzing maternal blood samples from full-term and preterm births, MacKenzie and her colleagues have found activation of the fetal immune system (specifically, T-cells and dendritic cells) in the preterm patients. It seems that in some cases, the fetal immune system raises an inflammatory response to antigens in the maternal blood stream. "What is it that makes preterm fetuses 'skip a grade' and develop their T-cells {the ones responsible for inflammation} early?" MacKenzie wonders. "And when this happens, do you call it an infection or do you call it changes in the microbiome?" She will explore this question as part of her research.

MacKenzie is also a pediatric and fetal surgeon, who entered this field with the aim of trying to treat birth defects. Her work on understanding the fetal immune response to maternal cells is relevant for treating blood disorders in fetuses, by transplanting healthy stem cells from the mother. She is currently testing this strategy in a clinical trial in patients with alpha thalassemia.

Kang Chen, at Wayne State University, also studies immune dysfunction in preterm birth, but he focuses on a different aspect of the immune system. In work supported in part by a 2015 Preterm Birth Research grant, Chen is elucidating what Advisory Committee chair Muglia calls "an entirely novel role for B-cells."

Researchers have long marveled at the way the human immune system combines the qualities of precision, versatility, and staying power, all managed with great economy of means. What makes this feat possible is a continuous chain of interactions between two of its main components: B-cells and T-cells. An important job of the B-cells is to produce antibodies, the germ-tagging proteins that patrol the body, whereas each type of T-cell has its own function, including helper T-cells, which help the B-cells make antibodies; cytotoxic T-cells, which destroy cells that have become infected or cancerous; and regulatory T-cells, which can turn off other immune cells.

"Research indicates that up to one-third of preterm labor is triggered by inflammation or infection," says Chen, "and we know that the mother is altruistic: she weakens her own immune system in order to tolerate the fetus." This accommodation by the maternal immune system is certainly necessary, but it can take a toll on the mother's health; for instance, a recent study of pregnant and non-pregnant women with influenza found the pregnant women showing signs of more severe illness. In the negotiation of immune tradeoffs between defending the pregnant body and supporting the pregnancy, Chen has identified a molecular go-between he calls progesterone-induced blocking factor 1, or PIBF 1. Late in pregnancy, the B-cells produce PIBF 1 with the purpose of suppressing inflammation, which could otherwise trigger preterm labor.

THE BIOMES OF PREGNANCY

As **Indira Mysorekar** surveys the multifactorial landscape of preterm birth research from her lab at Washington University in St. Louis, the question that intrigues her is: Which factors are more important than others?

"People tend to focus on infectious disease and inflammation, partly because it's much more definable than some other causes and can be more easily studied," she says. But conventional notions of "infection" have changed considerably in the past decade, and Mysorekar has played a significant part in that change. Now a professor of obstetrics and gynecology, she is "a perfect example of how this grant made a difference in her career," says committee advisor Jerome Strauss. "We were just in the right place at the right time with the right grant program."

When Mysorekar was awarded her grant, in the first year of the Preterm Birth Research Initiative, BWF was at the forefront of bringing people in from outside the usual channels of preterm birth research. "And I was about as outside the field as possible—I was studying urinary tract infections, so I came in with a completely different mindset," she says. Her different frame of reference allowed Mysorekar to see a piece of the puzzle that had not been detected before.

"It turns out there is a placental microbiome, so the womb is not sterile, as had been thought," she goes on. "Before this, any indication of any microbe had been considered an infection. Now these are not considered pathogenic 'reservoirs' of bacteria any more; now we know are likely part of the normal microbiome, the microbial community that lives in every organism."

In fact, Mysorekar continues, "Not only is there a placental microbiome, there's also a fetal microbiome. We now understand that the microbiome is an important part of us and is even as important as our genome. It's no longer a question of being infected or even colonized—it's more a question of being exposed," in the same way that, to the receptive mind, exposure to new facts and new ways of thinking can represent an education. With preterm birth, though, it appears that "the fetal immune education is not complete," and this incompleteness may be responsible for some of the problems in preterm babies. "When you get new knowledge, you have to change paradigms," says Mysorekar. "Doing so makes your explanation more complicated, but that just means it's more realistic."

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UNUSUAL IDEAS WELCOME HERE

This willingness to consider new, more complicated analyses is central to the mission of the BWF Preterm Birth Initiative. As Nelson observes, "When it comes to something like [the physiology of pregnancy], which is critical to the survival of the species, it makes sense that there would be a lot of checks and balances."

Hence, the PTBI continues to cast a wide net. Advisory committee member Susan Fisher says, "I think we get and fund the best ideas that are out there, so there are no glaring holes" in the BWF-supported coverage of preterm birth research. Recent awardee Laura Ensign would encourage interested researchers to consider initiatives beyond the boundaries of standard grant applications. "For anyone who feels like they're too outside [this field] or that their research really doesn't fit into the preterm birth community, don't rule out the possibility that you may have something to contribute," she says. Indira Mysorekar, from the first year of the program, describes herself as still "extremely excited," adding, "I'll always be grateful to BWF for supporting me when I had these unconventional ideas."

No one who studies preterm birth would deny that this young field of research faces many challenges—but some challenges, if viewed from a certain perspective, can look almost like invitations. Says Strauss: "Clearly, given the complexity of the problem, there are opportunities for people with a variety of backgrounds and skill sets. It's a pretty big tent; what's wonderful about BWF is that it's much more ecumenical in its search for new talent and fresh thinking. That's a great success story, and something that does not often happen."

THREE ENGINEERING PERSPECTIVES

Considered as a distinct area of research with its own characteristics and challenges, preterm birth is a very young field—so much so, says Whitsett, that "I think we're just now beginning to realize the environmental, genetic, social, and biological factors contributing to the complexity of it." Fellow advisor Strauss agrees, comparing the current state of the field to a popular three-dimensional puzzle: "This is a complicated Rubik's cube," he says. "We're beginning to understand just what the pieces are, but fitting them together in the right order won't be easy."

Some of the most innovative approaches to the puzzle have come from the seemingly unrelated field of engineering. A prime example is the work of **Mala Mahendroo**, at the University of Texas Southwestern Medical Center, who was awarded a grant in the first cycle of the Preterm Birth Research Initiative funding, in 2009. "I study how the cervix transforms from a closed and rigid structure to a flexible and open one, then repairs itself afterwards," she says. Using mice (in whom the process is quite similar) as her primary animal model, Mahendroo seeks to understand how this transformation takes place in a normal, full-term birth and how the process goes awry in a preterm birth.

One part of the cervix that undergoes dramatic structural changes in the course of pregnancy is the extracellular matrix (ECM), made up of interwoven fibers of collagen and the gel-like substance known as hyaluronic acid. "You can think of the ECM like a rope whose fibers are really tightly woven together, so the rope is very stiff," says Mahendroo. In the late stages of pregnancy "the fibers of the rope are all still there," she adds, "but "now they're very loosely interwoven, so the rope is much more pliable." Exactly how the steroid hormones of pregnancy produce this effect on the collagen fibers is currently the focus of her research.

Mahendroo found her way to this line of investigation after training as a molecular biologist and biochemist, with an interest in reproductive biology. She now collaborates on some studies with fellow PTBI awardee **Laura Ensign**, a biomedical engineer at Johns Hopkins School of Medicine. In her work with Mahendroo, Ensign is focusing on the epithelium, the thin layer of tissue that lines the cervix and shields it against "ascending infection" from the vagina; such infections during pregnancy are often associated with preterm birth.

Ensign holds faculty appointments in departments ranging from ophthalmology to gynecology to pharmacology and molecular sciences, all converging in the area of nanomedicine. Her lab studies the biological barriers—such as hyaluronic acid, mucus, and so on—that the body produces both in health and in disease. Ensign and her colleagues have developed a number of nanoparticles for drug delivery, which may offer advantages over other options such as microdelivery or drug-eluting implants. This is where "the engineering part" comes in, she says: "We create 'containers' for drugs so we can provide sustained drug delivery directly where it's needed." The benefits include greater efficiency, with less medication wasted elsewhere, and possibly fewer or milder side effects.

The drugs that these nanoparticles will contain are, for the most part, in development. According to Ensign, up to now there have been few treatments approved for preterm birth, or even used off-label to good effect, and all so far have been based on progesterone. Although more options are badly needed, a good deal of testing must take place even before any of these reach the stage of formal clinical trials. Ensign sees her role as "offering a toolkit so that if other specialists say, 'We should be looking at X [drug or therapeutic intervention], they have the means to test it in a controlled way and to get some solid information."

In her lab at Johns Hopkins, Ensign and her collaborators have increased the information-yield of each experiment by running testing on two parallel tracks. Ensign explains, "Along with drug delivery in the mouse model, we administer the same drug to samples of myometrial tissue [the smooth-muscle tissue in the uterus that contracts during labor] that we obtain from planned caesarean sections at the JHMI hospital. These samples can tell us a lot, because they're myometrial tissue from women who are full-term but aren't in labor—which, by the way, is a very pro-inflammatory process that has its own effects on the tissue. It's an easy and a very low-impact way to get human tissue, with the patient's full consent, of course, after the C-section and right before closing up. The way we do our research, because we're closely associated with the hospital, we just naturally think that way."

Kimani Toussaint, at the University of Illinois at Urbana-Champaign, begins a discussion of his research on preterm birth by admitting he has been somewhat surprised to find himself investigating a topic that seems so far removed from his academic background. "I'm an electrical engineer by training and in the department of mechanical science and engineering at my university," he says; moreover, he's affiliated with the departments of electrical & computer engineering and of bioengineering, as well as the Beckman Institute for Advanced Science and Technology. Then again, when it comes to assessing a situation of complex risk that combines an unknown number of variables, who better than an engineer with multiple areas of expertise? Perhaps it's not so surprising after all that Toussaint received PTBI grant in 2017 for his innovative approach to estimating the risk of preterm birth.

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Although the incidence of preterm birth hovers around 10 percent in industrialized nations, the likelihood of a preterm birth for any particular pregnancy is frustratingly hard to gauge. It's well known that women with a short cervix (less than about 2.5 cm) have an increased risk of giving birth prematurely, but this indicator is far from foolproof; many women with a short cervix carry their pregnancies to term. Therefore, says Toussaint, "We need a more accurate way to predict preterm birth." He is developing just that, combining the information gathered by two different techniques to come up with more nuanced, more accurate risk assessments than had been available before.

One technique is quantitative second-harmonic generation (SHG) microscopy, a means of three-dimensional imaging that makes it possible to analyze the way collagen is reorganized, or remodeled, in the cervix. Collaborating with Toussaint are two researchers at the University of Illinois, Amy Wagoner Johnson in Champaign-Urbana and Barbara McFarlin in Chicago; McFarlin has been able to use quantitative ultrasound to correlate distinct states of cervical remodeling with successive stages of gestation. The challenge is that quantitative ultrasound does not provide information on exactly how the structural organization is changing with gestational state. This is why Toussaint and his team want to combine technologies. SHG imaging would provide three-dimensional information on the cervical collagen structure and how it remodels. This knowledge could help to improve models for understanding the onset of spontaneous preterm birth, which will ultimately contribute to better methods for predicting the risk of preterm birth as well as to new prospects for treatment.

But Toussaint would like to see still more. "Even though quantitative ultrasound provides a good signal with strong positive predictive value, we still don't know at what point of collagen remodeling spontaneous preterm birth is triggered, and why it occurs only in some women with short cervixes but not others," he says. "So the question now is, Can we correlate collagen structural organization with mechanical properties?"

Investigating the mechanical properties of the cervix is the job of another technique developed by Toussaint's colleague Wagoner Johnson: A mechanical probe is used to apply a small force on cervical tissue, and the response of the tissue to the applied force provides information on the stiffness of the cervix.

"This is where out-of-the-box collaboration comes in," says Toussaint. "Nano-indentation doesn't tell you about structural organization, but it can give you the mechanical properties. Meanwhile, second-harmonic-generation microscopy doesn't tell you about mechanical properties, but can give you information on structural organization." The next challenge is figuring out how to calibrate the two different kinds of data to yield clinically useful information. Toussaint and his collaborators are up for it: "We're still very much in the initial stages—we're just beginning," he says. This work exemplifies the kind of scientific creativity that the Preterm Birth Research Initiative aims to support. As Advisory Committee chair Louis Muglia puts it, "We want to be able to foster new ideas. We're not model-centric or technology-centric, we really want to see things that will make us think differently."

OBSERVATIONS FROM THE ADVISORY COMMITTEE

In its first five grant cycles, the BWF Preterm Birth Research Initiative has supported projects from across the scientific spectrum. While a PTBI grant represents a unique opportunity to each awardee, the overall initiative is aimed at filling a single significant gap in biomedical research. "Reproductive science in general is an area we consider underfunded and undervalued," says senior program officer Rolly Simpson.

The recipients of PTBI grants are selected by the Advisory Committee, who meet to read and discuss applications and to interview candidates. Susan Fisher, a member of the committee, says, "The application review is a very fair process with a lot of open discussion, and we're given sufficient time to debate the merits of each proposal." Reviewers are assigned primary or secondary roles, but the committee as a whole votes on each proposal. In a typical grant cycle the committee is likely to screen about applications from about three dozen researchers, of whom about 20 are then asked to submit a full grant proposal. From this group, 10 finalists are invited to the BWF offices to give a presentation of their research and have an interview with the Advisory Committee, and ultimately five or six applicants are selected to receive one of the four-year grants.

Members of the Advisory Committee are researchers of high standing in their own fields, says Simpson, adding, "We look for people who have a great diversity in their areas of expertise." Jeffrey Whitsett, who has served on the committee from the time it was first formed in 2009, says his fellow members are "very creative people with novel ideas and the broad perspective needed to see the potential impact of the diverse scientific issues underlying preterm birth."

For Fisher, serving on this committee counts as serious fun: because the focus is on preterm birth, whose causes for the most part are not well understood, she says, "Most of the applications we receive, almost by definition, are very innovative." Michael Nelson confirms this: "The approach of 'Let's collect some more cells in the same way' is not what we're looking for."

In casting such a large and wide-open net, the Preterm Birth Research Initiative creates a special context for the interested research community. Whitsett notes that the program encourages applications from people with very diverse backgrounds, who may not be familiar with the latest developments in one another's fields. A benefit of the PTBI that goes beyond the financial support, he says, is the development of a growing network of enthusiastic scientists, who bring their own areas of expertise to the problem of preterm birth at the gatherings sponsored by BWF. "Where else would you get perinatologists, pediatricians, and obstetricians sitting next to biomechanical engineers, geneticists, and embryologists, chatting together with a physicist interested in mucous?" he asks rhetorically.

The scientists who gather within this context vary widely not only in their specialized fields but also in their years of experience. An application from a newly appointed associate professor receives the same consideration as one from a senior scientist; the most important criteria are the freshness and utility of a proposed line of investigation and the degree to which it brings in collaborators from other disciplines. Says Simpson, "One of the things we particularly look for is collaborations, because we feel that often the best science is done at the interface of different fields. You've got to be willing to work outside your silo." Louis Muglia , chair of the Advisory Committee, adds, "We're not taking people before [they have] a PhD, but we are very interested in taking people toward the end of their

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postdoc appointment." He makes a point of encouraging researchers to apply even if they haven't yet completed their postdoctoral work, so that they can "hit the ground running" with BWF support when the postdoctoral funding ends, rather than finding themselves stranded. Besides, he says, "It always feels very good to award a grant to people who are early in their career and who you are betting on to make a real contribution to the field."

Nelson sounds an encouraging note as well. "If you've got a novel idea and some preliminary data to support it, this is a good place to apply," he says. "And if there's any question about whether it's suitable, you can always get in touch with an administrator of the program"—for instance, Rolly Simpson, who can give insight into the "between-the-lines" message of the program description and application. According to Nelson, Simpson is also good at putting people in touch with potential collaborators on a project. Moreover, Simpson can be a resource when it comes to revising a hypothesis, whether to sharpen the focus or to bring it more in line with what BWF is looking for. "The application is not an endpoint, it's a process," Nelson emphasizes.

Laura Ensign is one of the newer PTBI awardees, but she has already formed a clear sense of the program that she would like to pass along to prospective applicants. She says, "Even though these grants are awarded for specific research projects (as opposed to a fellowship, which invests in the person), you really want to think about where this is going to take you in your research, what will you do next, how are you going to be the person who will break open the field? My impression is, that's something that BWF really wants to hear. They're investing in your project, but they're also investing in you. I've had so many useful conversations and gotten so many collaborations as a result of these meetings—honestly, it's been a lot of fun."

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