



March of Dimes Prematurity Research Center Stanford University

*The nation's first transdisciplinary research center dedicated to identifying
the causes of preterm birth*

Newsletter: Issue 02, Spring 2016

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Greetings

Here at Stanford University, we are beginning to embark on performing deeper, more associative analyses. While we continue to investigate what pregnancy looks like at different points during gestation – What [microbes are present](#)? What [genes are expressed](#)? What [distinctive immune features](#) characterize women with a history of term versus preterm birth? – the next step is to explore functions and interactions. Entering year six, we are integrating multiple data sets taken from different sources and performing correlations, looking for hallmarks of preterm birth. We are eager to receive data from other March of Dimes institutions so we can combine our efforts and report findings of greater strength.

On March 14 and 15 we reviewed our progress with researchers from the four other March of Dimes Prematurity Research Centers and March of Dimes scientific leadership. Dr. David Relman presented his latest work using whole genome shotgun sequencing to explore the interaction of microbes with phages, since host-phage dynamics may impact gene expression. We also unveiled our elastic net analytic algorithm to link clinical data and phenotypes, and reviewed analysis of lymphocytes in peripheral circulation via single cell mass cytometry by time of flight (CyTOF) to characterize gestational age. Our goal in creating these relational networks is to find which measures have the greatest influence in determining preterm birth.

In this issue, we speak with Dr. Stephen Quake about his transcriptomic work for Theme 3, (page 3). In 2014, Dr. Quake and his team released a paper that showed they were able to longitudinally track the phenotypic expression of pregnancy in both mom and fetus. This analysis continues today thanks to ongoing sample collection from our Prematurity Research Center cohort. Additionally, Dr. Quake's lab is investigating cervical remodeling during birth in a smaller, more exploratory project. Later this year, we are planning to host a webinar where Dr. Quake can present his latest findings. We look forward to validating a transcriptome signature for preterm birth as well as the transcriptomic characterization of gestation – a novel way of dating pregnancy. Our hope is that Dr. Quake's data will correlate with Dr. Relman's group Theme 1 work aimed at identifying gene pathways by analyzing host-phage dynamics of the microbiome. They should also correlate with CyTOF work, where we are looking at time-dependent changes of the immune system during pregnancy.

We hope you enjoy reading our spring 2016 issue and look forward to your feedback.

What's inside?

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IN BRIEF: Review the latest publications and our leadership. Our principal investigator, Dr. David Stevenson, is honored with the Joseph W. St. Geme, Jr. Leadership Award.

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GROWING COHORT: Since 2012, we have enrolled over 300 participants in our Prematurity Research Center cohort. Our on-site supervisor and a participant explain what it's like to be involved in such a time-intensive clinical research study.

Leadership

Principal Investigator: David Stevenson, MD



Co-Principal Investigators:

Gary Darmstadt, MD

Maurice Druzin, MD

Gary Shaw, DrPH

Paul Wise, MD, MPH



Theme 1: The Microbiome and Preterm Birth

Lead Investigator: David Relman, MD

Theme 2: The Gene-Environment and Preterm Birth

Lead Investigator: Atul Butte, MD, PhD

Theme 3: The Transcriptome and Preterm Birth

Lead Investigator: Stephen Quake, DPhil

Analytics Leader: Jeff Gould, MD

Administrative Director: Cele Quaintance

Program Manager: Zaida Esquivel

Administrative Coordinator: Sarah Kramer

Newsletter Writer/Editor: Laura Hedli

march for babies®

May 1 is the [March for Babies, Silicon Valley 2016](#). Funds raised for this 3-mile, family-friendly walk help mothers have healthy, full-term pregnancies and support ongoing research efforts to solve the mystery of preterm birth.

Registration: 8:00 a.m.

Start time: 9:00 a.m.

1650 Senter Road
San Jose, CA 95112

Faculty Highlights

Dr. David Stevenson is the recipient of the 2016 Joseph W. St. Geme, Jr. Leadership Award by the Federation of Pediatric Organizations. The prestigious award recognizes Dr. Stevenson's longstanding commitment to caring for premature and high-risk infants, his dedication to mentoring researchers and trainees, and his investigation of the causes of premature birth in conjunction with the March of Dimes. Dr. Stevenson will accept the award on April 30 at the Pediatric Academic Societies meeting in Baltimore, MD. He serves as the Stanford University School of Medicine's senior associate dean for maternal and child health and is the principal investigator for our Prematurity Research Center.

On April 11, **Dr. Gary Darmstadt** was inducted into the Johns Hopkins Society of Scholars. Established in 1967, the Society of Scholars honors distinguished former Johns Hopkins postdoctoral fellows and faculty. Before coming to Stanford, Dr. Darmstadt began his career at Johns Hopkins as a resident and later served as the founding director of the International Center for Advancing Neonatal Health at the Johns Hopkins Bloomberg School of Public Health.

Recent Publications

Gaudilliere B, Ganio EA, Tingle M, Lancero HL, Fragiadakis GK, Baca QJ, Aghaepour N, Wong RJ, Quaintance C, El-Sayed YY, Shaw GM, Lewis DB, Stevenson DK, Nolan GP, Angst MS. [Implementing Mass Cytometry at the Bedside to Study the Immunological Basis of Human Diseases: Distinctive Immune Features in Patients with a History of Term or Preterm Birth](#). *Cytometry A* 2015, 87(9): 817-829.

Gage S, Kan P, Lee HC, Gould JB, Stevenson DK, Shaw GM, O'Brodovich HM. [Maternal Asthma, Preterm Birth, and Risk of Bronchopulmonary Dysplasia](#). *J Pediatr* 2015, 167(4): 875-880 e871.

Ying L, Becard M, Lyell D, Han X, Shortliffe L, Husted CI, Alvira CM, Cornfield DN. [The transient receptor potential vanilloid 4 channel modulates uterine tone during pregnancy](#). *Sci Transl Med* 2015, 7(319): 319ra204.

Q&A with Stephen Quake

*Stephen Quake, DPhil, has an impressive track record in rapid-genome sequencing. In 2008, Dr. Quake and his team published a paper in the **Proceedings of the National Academy of Sciences** about a new technique for diagnosing Down syndrome and other aneuploidies by analyzing DNA fragments present in mom's blood plasma. The technique works because fetal DNA presents in maternal blood early on during pregnancy, and the test is now regularly used in clinical practice by analyzing blood collected during a routine trimester visit. Dr. Quake also made national news when he had his entire genome sequenced for less than \$50,000 using technology developed in his lab. He was one of the first to enter a new age of personalized health care, where clinical recommendations are based on cumulative genetic risk assessment.*



Stephen Quake, PhD
*Professor of Engineering,
Bioengineering and
Applied Physics*

Lately, Dr. Quake and his lab are turning their attention toward the next logical piece of the human biology puzzle – RNA and transcriptome sequencing. Dr. Quake is our Theme 3 leader, investigating cell-free RNA in the blood of pregnant women to learn about variations that exist in the transcriptome and may be associated with early spontaneous preterm birth. In other words, what's actually happening in real-time to both mom and baby?

*In 2014, **Proceedings of the National Academy of Sciences** featured another paper by Dr. Quake and his team that analyzed the trimester blood samples from 11 pregnant women enrolled in our Prematurity Research Center cohort. Even with this small sample, they were able to demonstrate specific longitudinal phenotypic changes in both the mother and the fetus and track the developmental program of the baby. Thanks to our ongoing clinical research recruitment efforts, there is much more data for Dr. Quake's group to analyze.*

Also underway is a smaller exploratory investigation of cervical remodeling. Via single-cell transcriptome analysis of hysterectomy tissue and also cells from cervical swabs, Dr. Quake and his team are hoping to find clues about preterm birth that may not be perceptible by analyzing a bulk of tissue. While this work is being done in the lab, the results could have a big effect on clinical practice. Cervical swabs may become a reliable, even preferable, alternative to 2D ultrasounds (the current gold standard) for investigating preterm labor or preterm premature rupture of membranes.

Recently, we sat down with Dr. Quake to talk about his work studying the transcriptome and preterm birth.

Q: What can you tell us about what you're finding in your analysis of the transcriptome?

A: I would say we spent a lot of time looking for signals of preterm birth in the RNA and found some promising ones, but as we followed them up, they haven't validated with the statistical confidence we'd like to see. So we're going to hold off on really talking about that until we have a larger cohort that will validate with proper statistical significance.

As far as what's the normal path of development, we've learned a ton. There is a bunch of novel, non-coding RNAs and circular RNAs that people never knew were associated with pregnancy. There are some from the baby, some from the mom. You can see very clear signals of the placenta developing, and that's going to maybe provide a route for us to explore some of these placental issues, like placenta previa abruptio.

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With respect to the cervical remodeling project, my group spent a decade developing the technology to do this. We do hundreds to thousands of single cells for a given experiment. When you can analyze individual cells, one at a time, and look at their transcriptomes, they're each perfectly pure representatives of whatever cell type they happen to be. By combining all the individual transcriptomes and seeing which are similar, you can kind of work out, de novo, what are the different cell types present and what are their roles. That gives you a much clearer picture of the biology.

Q: And you would expect these cell types to be present no matter if mom has a normal birth or delivers preterm?

A: Yes. Their relative roles would be changing, we're hoping, when labor starts. We're trying to glean the molecular mechanisms there. So the thought is that if you're in the doctor the week before, and things are starting to remodel and you haven't felt it yet, then you might be able to pick it up.

Q: So then you could do something therapeutically to delay birth?

A: Or just be prepared, just go to the hospital and be ready. [The] same thing [is true] with the blood test. There are going to be things happening with the placenta that are either on program or not. You know, there's inflammation in the mother. You might see signals of that from a blood test that would [indicate that the] baby's coming out a little ahead of schedule.

Q: What's unique about this March of Dimes collaboration compared to other joint projects you've worked on in the past?

A: It's great because we run the whole gamut from basic science to clinical application. When we find stuff and go in [for our MOD meetings] to talk about it, sometimes the clinical significance is really not apparent to us. But the docs will say: "Oh, that would be incredibly useful for x, y, z." With Relman, specifically, we independently found ways to measure the microbiome from the cell-free DNA in the blood. And in trying to figure out how to validate which body sites are represented, we realized this cohort was great for that. We've had a really nice collaboration where we mash up direct swabbing with blood-based measurements.

Spotlight: Growing Cohort

Over 300 participants collect 150,000 samples to help us understand pregnancy and preterm birth

In our inaugural newsletter, we featured a Q&A with Dr. David Relman, who is one of our lead investigators exploring the microbiome and preterm birth. In order to track the changes in the microbiome during pregnancy, Dr. Relman and his team are analyzing data from hundreds of thousands of samples collected from women at the same points in time during gestation. Other investigators are tracking measurements from the same cohort, performing transcriptome (see p. 3) or mass cytometry analysis. The Prematurity Research Center cohort is comprised of our dedicated group of clinical study participants, and the collection process is part of their weekly routine. Here, we explore that process and what it entails. It begins with a brief conversation between clinical recruitment coordinator and potential participant during an intake exam at our maternal-fetal medicine and obstetrics clinics. What happens next can lead to a two-year relationship between parties.



Collection materials provided weekly to participants

Anna Stathopoulos arrived at Stanford's OB clinic for her intake exam just one week before the end of her first trimester. Pregnant with her third child, Stathopoulos wasn't concerned. She had already read the literature on "what to expect when you're expecting," and by now she felt she knew her body. Both of her daughters had been born at term, so prematurity was not a topic that crossed her mind with any regularity. When clinical research coordinator Ana Campos approached her about whether she wanted to enroll as a participant in a March of Dimes research study, Stathopoulos turned to her husband, an ER and ICU doctor, for his thoughts. She says the decision was an easy one for them to make.

“I understand how hard it is to get subjects sometimes, and the process. We believe in clinical research as a family, so why not participate?” says Stathopoulos, whose husband has done research on cardiac arrest, hypertension, and traumatic brain injury. “Obviously, I would love not to have a preterm baby, but I’m not looking into it any further or any less because of this project.”

What’s important is that potential participants know that they are not being targeted because clinicians expect they will deliver preterm. Nurses in the OB clinic explain that coordinators approach all new patients no matter their history. It’s just as critical – and perhaps even a prerequisite – to understand what characterizes a normal term pregnancy. For women who deliver preterm, it’s the same process, shifted to an earlier point during gestation. Why might that happen? Performing a variety of analyses on this cohort may provide clues. By correlating many measures at different points in time, researchers aim to find which measures – taken together or separately, if certain factors prove more predictive – may lead to premature birth.

On-site supervisor Olivia Tigre likes to stress to potential participants that even though this is called a “clinical research study,” there is no intervention or treatment. Rather, the goal is to observe the changes that occur over time and this requires precise tracking beginning, ideally, before 12 weeks into a woman’s pregnancy.



Clinical research coordinator Ana Campos (left) and on-site supervisor Olivia Tigre; in the background, the collection bags picked up from participants each week by our courier

“I really believe that what the investigators are doing can create meaningful results for future times, especially in other countries where women don’t get the prenatal care that we do here,” says Tigre. She warmly greets each patient and their partner (if present), offering congratulations first instead of immediately rattling off the objectives of the study. And if this sounds like a given when it comes to introductions, it’s taken some practice. Tigre had to learn how to balance meeting these moms-to-be for the first time with delivering her elevator pitch. It has to happen in the span of about two minutes, which is all the time she has with patients to gauge their interest in the study. Coordinators will often return to consent a new patient at the end of her OB intake exam, or will follow up at a later date to explain the study in more detail. Without a doubt, it’s a tough sell.

Each week leading up to delivery participants rub swabs at their elbow crease and behind the ear to collect bacteria on the skin. They swab to collect gum line, stool, and vaginal bacterial samples, and provide urine and saliva specimens too. Blood is drawn at trimester visits in the OB clinic and also at the time of delivery – from mom and baby as well as the umbilical cord. Participants then provide samples once a month for a year postpartum.

“Vaginal swabs and stool are the ones where I get the eyebrow look, like *What?!* When we go into detail about explaining to them what exactly it is that we need, it starts to get rid of some of their worries,” says Tigre, who is a study participant herself, having welcomed her first child last November. She uses this as an entry point when she introduces the methodology, and offers an instructional guide with steps detailing the collection process that participants can take home.

Stathopoulos found the instructions to be straightforward. “It took me about 10 extra minutes the first time doing it, and now it’s so routine that it’s part of my Tuesday nights,” she says. “I don’t think anyone likes the idea of swabbing every body part, but you get used to it. It really is not invasive in my opinion.” Each Wednesday morning Stathopoulos leaves a bag for the study’s courier, who travels around the Bay area – as far north as San Bruno and as far south as Los Gatos – to collect samples.

Senior research scientist and lab director Dr. Ron Wong explains that this is one of the first studies to begin frequent biospecimen collections so early on in a woman’s pregnancy. And the methodology has served the science – Dr. Relman’s analysis of the microbiome has indicated noticeable changes in the microbiome that occur sometime at the start of the second trimester. As a result, coordinators may only enroll participants during the first trimester, and Tigre says, on average, women are consented at 9 or 10 weeks into their pregnancy

For their efforts, participants are given gift cards throughout the study – when they sign the consent form at their intake exam, at each trimester visit thereafter, and shortly after giving birth. They may receive up to \$350. Coordinators stay in contact with participants, sending weekly updates (usually via text message) for sample collection and check in in-person at their monthly OB clinic visits.

Despite the challenges inherent in the process, the recruitment coordinators have been able to enroll over 302 participants since the study began data collection in 2012. Of that total, 193 delivered and 26 delivered preterm (13.5%). Tigre estimates that they add six or seven new participants each month and at least 70 per year. Study compliance rate is at nearly 90 percent, which is remarkable for a research effort that relies on its participants to contribute so much.

Dr. Wong reports that at the start of April 2016, we have approximately 65,000 unique samples and 150,000 total samples with 81 samples per box – all told taking up six freezers of storage space. Each sample has to be labeled appropriately with the right barcode and entered into Redcap, a HIPAA-compliant database used for storing research data here at Stanford. Because samples are now being collected from multiple centers, this makes data entry and collation especially challenging.

With this massive influx of data, processing cannot happen immediately upon the receipt of a sample to the lab. That said, coordinators tell participants that their healthcare provider will be notified if investigators detect any abnormalities throughout their pregnancy.

“I was hoping if something goes wrong, because I’m considered a normal pregnancy, that this is like a double check of everything and we catch it sooner than later,” says Stathopoulos, who expects to deliver her third daughter at Lucile Packard Children’s Hospital Stanford in mid May. “It’s good to know that more people are on my side.”

Contact Us

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