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 03, Fall 2016

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Stanford Children's Health | Lucile Packard Children's Hospital Stanford

What's inside?

Greetings

Summer was a busy time for our Center. In June we released our annual report, and in August we conducted our annual site visit. There, we featured presentations highlighting our three areas of inquiry. Dr. Steve Quake described the transcriptomic clock, showing how gene expression patterns change over the course of gestation. Dr. David Relman described additional studies that validated our previous observations about the microbiome and revealed features predictive of preterm birth for Caucasians. Complementing both the transcriptome and microbiome work, Dr. Brice Gaudilliere presented on the immunome. While Dr. Atul Butte left Stanford last year to take a position at University of California San Francisco, he remains an integral part of our Center's operations. During the site visit, Dr. Butte described his group's work on the gene-environment area. He also provided updates on the development of the data repository for the entire March of Dimes Prematurity Research Center network with input from all five centers.

Indeed, we are putting more emphasis on integration. Up until this point, mainly, we have reported on our different areas of inquiry. Now, with Dr. Nima Aghaeepour's use of the weighted elastic net and other approaches for integrating different types of data from a variety of sources, we have at our fingertips powerful computational strategies that allow us to discover signatures of preterm birth. At once we can exploit measurements from the transcriptome, microbiome, proteome, cell-signaling behavior using CyTOF technologies, and the metabolome. We're finding that the relationships among the different measurements are most important. We have also developed an immune clock that characterizes the immune system across gestation.

Taking a more macro view may very well be the theme of this issue's content. Rather than focus on the scientific underpinnings of preterm birth as we have in past issues, we wanted to tell you more about our Center's work in epidemiology and global health. We are committed to investigating what factors increase risk for delivering preterm (see Dr. Suzan Carmichael's research and the Analytics sidebar, pages 2-4) and ensuring essential health services are provided to women and children in areas of unstable governance and political unrest. Dr. Paul Wise presents two case studies, one from Guatemala, another from the Democratic Republic of Congo, and uses these to illustrate the collaborative efforts we are pursuing with others on the Stanford campus to help those most in need (pages 5 and 6).

Thank you for reading, and we look forward to hearing your thoughts.

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 Transcriptome and Preterm Birth
Lead Investigator: Stephen Quake, DPhil

Theme 3: Bioinformatics
Lead Investigator: Atul Butte, MD, PhD

Administrative Director: Cele Quaintance
Program Manager: Zaida Esquivel
Administrative Coordinator: Sarah Kramer
Newsletter Writer/Editor: Laura Hedli

Q&A with Suzan Carmichael

Suzan Carmichael, PhD

Professor of Pediatrics, Neonatal and Developmental Medicine

Suzan Carmichael, PhD, focuses on epidemiologic studies related to maternal and child health. Her PhD dissertation was on preterm delivery and maternal weight gain, after which she went on to investigate risk factors for birth defects, working for the Centers for Disease Control and Prevention and later the California Birth Defects Monitoring Program. The commonality in all her work has been trying to find ways to improve newborn and maternal health, in particular looking at nutritional, environmental, and genetic risk factors.

Recently, we met with Dr. Carmichael to talk about her work that is part of a larger center-wide exploration of the underlying reasons for disparate preterm birth occurrences in the population with attendant deep dives into both biologic and social explanations.

Q: Your research with the March of Dimes explores the epidemiology of preterm birth. That's a broad topic. What issues are you currently investigating?
A: Within the context of the Center, one of the key issues that I'm focusing on is disparity. For example, for a long time we've known that black women are at least twice as likely to have a preterm delivery as white women. In fact, they are 3 times more likely to have a very early preterm delivery (<32 weeks), and these are the babies most at risk of dying and having long-term health problems. We don't know why these disparities exist. But if we did, it would likely help us understand preterm delivery in general because whatever is driving that race disparity probably drives risk in other ways as well, among individuals and at the population level.

Q: How are you investigating race and preterm birth rates?
A: We have taken all births in California for many years and geocoded the mothers' addresses. Based on knowing where they lived when they had their babies, we can characterize their neighborhoods using information from other data sources like the U.S. census. We can look at a compendium of variables, starting with variables from the census that characterize the neighborhoods. What's the rate of poverty? What's the education level? Unemployment level? Household income? This could be overall for the entire neighborhood, but also, what do those parameters look like among, for example, blacks or whites? Even among women versus men? How disparate are they? All of these are aspects of social context

and social disadvantage. We're looking for unique ways to analyze these types of variables to really understand what may be driving these disparities that we see.

Q: In your first-authored paper "Population-Level Correlates of Preterm Delivery among Black and White Women in the U.S.," you explored geographic variability in the prevalence of preterm delivery using county-level sociodemographics, socioeconomic level and environmental exposures. You found variability in preterm delivery could be explained by these population-level risk factors, but more so for whites than blacks. How do the variables you're defining in this new project differ?
A: That paper with Mark Cullen and others looked at factors associated with prevalence of preterm delivery as opposed to individual-level risk. What we're doing in California is at the census-tract level, which is much smaller than a county. It may be 5-6,000 people on average in a tract versus the county, which could be all of L.A. county.

Q: You emphasize the importance of studying both individual- and population-level risk. How can we make sense of these distinctions in practice?
A: Often, we focus on the individual level, but it's important to focus on both if we really want to think about how to be best informed about what's going to work as far as policies and interventions that are developed, whether they be at an individual level or at a population level.

For example, folic acid fortification. We tried interventions more targeted toward an individual behavior, which was to try to get women to take a folic acid-containing supplement. The percent of women taking vitamins didn't really budge much after policies were advocated and articulated. So then we turned to fortification of grains in the food supply. It was a population-level intervention because an individual-level intervention wasn't going to be as cost effective, or perhaps not as effective. Maybe at a population level you're not able to increase the intake of the individual as much, but you may have more of an impact because you are reaching more people.

We need to try to keep those approaches – individual versus population – in some sort of balance. I think we get out of balance and we consider them in separation too often.

Q: Speaking of separation, you were interviewed by NPR about our nation's stagnant, persistent rates of stillbirth (1 for every 160 births) and how we study stillbirth independent from preterm births. Another paper you first-authored in 2015 found increasing obesity (based on BMI classifications) to be associated with greater risk of stillbirth, with somewhat stronger risks being observed among nulliparous women than parous women for the earliest stillbirths. You noted this finding parallels earlier associations between obesity and preterm births in nulliparous women.
A: At the crux of this is the importance of studying stillbirth when you're studying preterm delivery. Stillbirths have declined, but it's been among the later stillbirths. "Later," here we can say, is 28 weeks or more. About 80 percent of all stillbirths occur at less than 28 weeks. The reduction in overall prevalence of stillbirth has been in the later stillbirths, and we think that's likely due to improvements in obstetric care.

If you look at all deliveries that occur from 20-25 weeks – stillbirths plus preterm deliveries – about 45 percent of those might be stillbirths. We're often focusing our studies on early preterm birth. But the point is, if we study those and we don't study stillbirths, we're really only studying a minority of all deliveries at that age. Based on what we know, they are likely to share some common etiologic pathways. I advocate for more cross-talk, more inclusivity in our studies rather than making these divisions so stark in how we look at outcomes.

Q: Yes, that cross-talk is important, and especially so, I imagine, for a transdisciplinary Prematurity Research Center. Is there somebody within our Center who's saying: "Great, this is what you found in your epidemiologic studies. Let's now explore the underlying biology"?
A: Yes, that's exactly what's the hope of the center is, that each arm – whether it be looking at the cellular level or the neighborhood level – that each arm will foster ideas in the other so that we can come at this difficult problem in multiple ways. How do those arms work together and spawn other studies? Definitely the conversation goes in all directions.

Our Center's Unique Analytic Resources ☑

Leveraging nearly 20 years worth of linked data on mom and baby

With approximately half a million births per year in California, our Prematurity Research Center has access to a tremendous amount of data – nearly 20 years worth of birth certificates linked with delivery hospitalization summaries. That's 10 to 11 million linked entries to date. The Center's Analytic Core is led by Drs. Gary Shaw and David Stevenson and enables investigators like Dr. Carmichael and others to interrogate these unique data resources with the help of highly experienced biostatisticians including Jonathan Mayo, MPH, Peggy Kan, MS, and John Oehlert, MS. These biostatisticians develop and implement our epidemiological analyses on preterm birth disparity. These resources have been made possible with funding from the Child Health Research Institute.

Mayo says that the wealth of "delivery hospitalization data are crucial as they contain ICD-9 codes that allow us to differentiate types of preterm birth and maternal medical conditions." The most recent year of complete linked data on file is 2011, with entries from 2012 coming soon. The data also includes information on infant mortality and stillbirths.

With the addition of new variables to the birth certificate – like details about mothers' birthplace and body mass index (BMI) – our biostatisticians are afforded more ways in which to analyze the data. Our Center has studied the effect of both obesity and underweight on preterm birth at the population level. We are currently working with the state's biobank repository of prenatal screening to obtain serum samples to examine potential biomarkers for preterm birth among women with high and low BMI.

While there is a declining rate of preterm birth in the state of California compared with other states (for 2014, the CA preterm birth rate was 8.3 percent compared to the national average of 9.6 percent), our investigators remain very interested in the data on early preterm births, or babies born at less than 32 weeks gestational age. Given the transdisciplanary nature of our group, our biostatisticians work with people from a variety of backgrounds – physicians, epidemiologists, residents or fellows – who all have questions about early preterm delivery that may be able to be answered by this data. "We try to leverage the large number of births in the state by examining maternal conditions of pregnancy and their association with preterm birth," says Mayo.

Recent Publications

Kowalewski L, Curtis M, Mitchell C, Rand L, Berns S, Shaw G, Stevenson D. California Summit on Preterm Birth. *Harvard Health Policy Review*. 2016;15;2, 33.

Ferrero DM, Larson J, Jacobsson B, Di Renzo GC, Norman JE, Martin JN, Jr., et al. Cross-Country Individual Participant Analysis of 4.1 Million Singleton Births in 5 Countries with Very High Human Development Index Confirms Known Associations but Provides No Biologic Explanation for 2/3 of All Preterm Births. *PloS one*. 2016; 11:e0162506.

Girsen AI, Mayo JA, Carmichael SL, Phibbs CS, Shachar BZ, Stevenson DK, et al. Women's prepregnancy underweight as a risk factor for preterm birth: a retrospective study. *British Journal of Obstetrics and Gynaecology*. 2016. Epub ahead of print.

Wallenstein MB, Shaw GM, Stevenson DK. Preterm Birth as a Calendar Event or Immunologic Anomaly. *JAMA Pediatrics*. 2016;170:525-6.

Wise PH. Child Poverty and the Promise of Human Capacity: Childhood as a Foundation for Healthy Aging. *Academic Pediatrics*. 2016;16:S37-45.

Zhao H, Kalish F, Wong RJ, Stevenson DK. Infiltration of myeloid cells in the pregnant uterus is affected by heme oxygenase-1. *Journal of Leukocyte Biology*. 2016. Epub ahead of print.

Fighting Premature Birth in the Most Dangerous Places on Earth

by Paul Wise

One of the most important attributes of the March of Dimes Prematurity Research Center (MOD PRC) at Stanford University is that it was created as a transdisciplinary network. While the Center has directed much of its activity at understanding the science of premature birth, it has also been involved with innovative, global efforts to address premature birth and related conditions in countries with political unrest and unstable governance. The following two cases depict the nature and scope of this challenge.

Paul Wise, MD, MPH

Professor of Pediatrics, Neonatal and Developmental Medicine;

Senior Fellow, Freeman Spogli Institute for International Studies

CASE 1:

When we first saw the baby, we knew something was terribly wrong. Even though she was almost three months old, the child was tiny, emaciated, and staring blankly into space. As the grandmother told the story, little "Princesa" was born two months prematurely in their small tin-roofed home, her mother having received no prenatal care. Because the cost of seeking medical care for the child was far beyond this family's capacity, it took five days before they could borrow the funds necessary to bring this child to a hospital. Our examination revealed that, in addition to suffering from profound malnutrition, Princesa was blind and probably deaf; both would likely have been prevented by immediate, appropriate care. The child was immediately entered into the Stanford-Tijax Young Child Nutrition program, a collaboration between Stanford and the local community health worker system. We all knew that in rural Guatemala, however, the challenges facing this child and her family were enormous. There is no health insurance. There is no early child development program. There is no special education. There are no social services. After years of civil war, widespread corruption, and devastatingly high levels of criminal violence, there remains no functioning health system in Guatemala for the vast majority of its citizens.

CASE 2:

It was at once one of the saddest and most inspiring sights I had ever encountered in my professional life. I had just toured the wards of Panzi Hospital, the primary medical facility in the war-torn eastern region of the Democratic Republic of Congo, when we emerged into a large, open courtyard filled with hundreds of women and children, some clustered in small groups, others tending to chores or walking quietly on their own. There were no men. I was told that the women had come to Panzi Hospital seeking refuge for they were the victims of rape and had been ostracized or displaced from their home communities. Many had been on the move for months, and many had given birth in the forests along the roads, without any health care for them or their newborns. Not surprisingly, both maternal and newborn mortality was catastrophically high.

These two cases, while depicting conditions in two very different parts of the world, reflect what has become the central challenge to global child health: The areas of the world in greatest need of essential women's and child health interventions are precisely those experiencing conflict, political instability and chronically weak governance. What is also not generally recognized is that premature birth is now the greatest killer of young children globally, and 14 of 15 countries with the highest newborn mortality rates in the world have been plagued by ongoing civil unrest and violent conflict. As the Ebola Virus outbreak in West Africa also revealed, the weakness of health systems in areas plagued by conflict and weak governance can also represent a threat to the larger international order.

In two recently published articles, my colleague Gary L. Darmstadt, another co-Director of the MOD PRC at Stanford University, and I have documented the importance of addressing premature birth, stillbirths, and neonatal mortality in areas of the world plagued by ongoing conflict and political instability. Current global maternal and child health programs and virtually all universities have largely avoided working in these areas despite overwhelming need. Given the logistical and security challenges inherent in working in these areas, this global reluctance is understandable. However, given the dramatic suffering in these areas coupled with our vast technical capabilities to prevent and treat maternal and perinatal health problems, we argue that this is no longer acceptable.

Members of the MOD PRC at Stanford University are partnering with colleagues across the Stanford campus to develop new strategies that bring together the technical capabilities of maternal and child health with the insights of political science and security studies focused on chronic civil conflict and weak governance. This effort, the *Children in Crisis Initiative,* has linked faculty in the MOD PRC, the School of Medicine and the Freeman Spogli Institute (FSI) for International Studies. We conduct urgently-needed research into the special obstacles health programs face in these areas. One of the first observations resulting from this collaboration was that each health intervention places distinct requirements on local political and security systems. For example, trauma reduction strategies demand much more of local governance systems than do newborn or immunization services. Significantly, this implies that we could make progress in delivering some of the most essential maternal and newborn health interventions long before more complete improvements in governance and security occurred. Rather, focused political strategies could allow the provision of these essential health services, even in very difficult security environments.

BEFORE

AFTER

The impact of the *NutriKas* app and Stanford-Tijax Young Child Nutrition Program is illustrated by the BEFORE and AFTER photographs of an infant born prematurely in Guatemala. (Photos with parental permission by Vicente Macario)

Another way we provide these essential services to women and children is through the development of new technological approaches specifically designed to overcome longstanding governance obstacles. For example, a collaboration between the MOD PRC, Department of Computer Sciences, School of Medicine, the Graduate School of Business, and FSI has led to the creation of a mobile app that overcomes the lack of nutrition and maternal and child health personnel in unstable areas of the world. Currently deployed in the highlands of Guatemala, the *NutriKas* mobile app has proven extremely successful in identifying malnourished children and facilitating the rapid provision of supplemental nutrition in areas without a functioning health system.

The MOD PRC at Stanford University has always had a deeply ambitious goal: to understand and ultimately eradicate premature birth around the world. To accomplish this will require not only new research and new clinical capabilities but also new strategies that ensure that these new capabilities will be provided equitably to all those in need. This means our goal is not only one of science but also of justice. It demands that we strive to reach the millions who live in areas of conflict, political instability, and weak governance. For all of us here, this means a sustained commitment to meeting the challenge of saving lives in some of the poorest and least secure places on earth.

Putting it together ⁂

In the last issue, we reported on the clinical recruitment efforts for the March of Dimes Prematurity Research Center cohort at Stanford University. The goal is to enroll up to 2000 participants who supply monthly and, at times, weekly samples. When specimens (such as swabs from skin, gums or vagina, or vials of blood) arrive to the lab on Tuesdays through Fridays, they are processed primarily by Adrian Yabut, Laboratory Assistant in the March of Dimes Prematurity Research Center Laboratory at Stanford University. Yabut barcodes each sample (check out the infographic), processes each specimen according to type, and then assigns each specimen aliquot a specific position in cryoboxes prior to storage at -80°C.

Says Ron Wong, Senior Research Scientist and Laboratory Director, "That was always an issue we had logistically: How can we do it? You can't store specimens in boxes based on each participant, otherwise you have a million boxes. So Adrian stores it by each sample type as they are received." For example, one box might be filled with skin or gum swabs, or plasma or serum, but from a variety of participants.

Even though each sample is barcoded using the same system, not every sample undergoes the same analysis. Whole blood may go to Dr. Brice Gaudilliere for CyTOF analyses, while swabs are exclusively for use in Dr. David Relman's microbiome study. Since the study has been ongoing for 5 years, many of the samples are out of the freezers and being transferred to different locations around campus.

Yabut can track a sample's location not only while it's in storage, but also when it has been transferred and undergoing analyses in the various collaborative laboratories. Following completion of analyses, the specimens are returned and Yabut rescans each sample, reassigning each to a specific position in cryoboxes prior to storage at -80°C. It's like a very complex puzzle that requires patience and precision to continually reconfigure the pieces. "We played around a lot, and we were fortunate because the study started slow," says Wong. "This system was set up by Alec Barlow and it's been pretty hearty, thanks to Alec's hard work on creating it. A lot of us take it for granted now."

All of the scanned barcode information is recorded in REDCap, an inventory tracker and data repository. Recently, a March of Dimes REDCap database has been established, where all five centers can share data thereby enhancing collaborative research efforts.

Contact Us

For more information on the March of Dimes Prematurity Research Center contact our Administrative Director, Cele Quaintance at: cele@stanford.edu
Or Writer/Editor Laura Hedli at: lhedli@stanford.edu

Visit us at: www.prematurityresearch.org/stanford/