March of Dimes Prematurity Research Center
Stanford University

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

Newsletter: Fall 2015

What's inside?

Greetings

Hello! We are proud to launch the inaugural issue of our March of Dimes Prematurity Research Center newsletter from Stanford University in Palo Alto, California. Here, our overall approach is to apply innovative, computational approaches to large and complex ("big"), linked databases in the State of California and elsewhere to discover new patterns of risk and causation of preterm birth. Our goal is to reduce the incidence of prematurity, currently at 1 in 8 births in the U.S. We also engage in discovery science, applying solutions for maximum health impact through the prevention of preterm birth and improving outcomes of babies born preterm. Transdisciplinary teams of investigators at Stanford explore three main areas of inquiry, and you can learn more about these on page 2. All told, we have over 130 doctors, researchers, and staff who actively participate in the Center's operations. In this newsletter, we share information about our current projects, most recent publications, and commentary from our investigators. We are pleased that the March of Dimes has written a feature highlighting our investigator, Ciaran Phibbs. To read about how preterm birth affected his family, click here. Thank you for your interest in our activities and investment in solving the mystery of preterm birth. We look forward to hearing your feedback.

Upcoming Events

SEPTEMBER 23: MOD Leadership Meeting (weekly)
OCTOBER 5: Abstracts due to Center PIs for the 2016 SRI Annual Scientific Meeting
NOVEMBER 17: World Prematurity Day

Please contact Ana Laborde at alaborde@stanford.edu if you would like more information on events.

Themes

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, PhD

Analytics Leader: Jeff Gould, MD

Administrative Director: Cele Quaintance
Program Manager: Ana Laborde
Administrative Coordinator: Sarah Kramer

1. ***The Microbiome and Preterm Birth:*** This area involves pre-pregnancy, pregnancy, and post-pregnancy maternal sampling from multiple body sites, characterizing the changes in microbial communities associated with normal and abnormal pregnancies, with a special focus on early spontaneous preterm birth. This work is supported by the Human Immune Monitoring Core and is closely linked with immunologic investigations exploring unique inflammatory signatures (mother and baby) associated with early spontaneous preterm birth, using traditional serum protein (metabolomics) analyses as well as single cell mass cytometry.
2. ***The Gene-Environment and Preterm Birth:*** This area is developing and applying bioinformatics methods to publically available genomic and environmental data to study the causes of preterm birth and develop novel diagnostics for early spontaneous preterm birth. The work is complemented by the development of novel computational methods to discover non-coding mutations most likely to contribute to early spontaneous preterm birth by finding the most promising candidate gene regulatory mutations.
3. ***The Transcriptome and Preterm Birth:*** This area complements the other two areas and is focused on characterization of the transcriptome of various phenotypes of preterm birth, providing putative RNA targets indicative of these pathologies.

Recent Publications

DiGiulio D, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DSA, Wong RJ, Shaw GM, Stevenson DK, Holmes SP, Relman DA. Temporal and Spatial Variation of the Human Microbiota During Pregnancy. *Proc Natl Acad Sci (USA)*. 2015; 112:11060-5.

Zhao H, Kalish F, Schulz S, Yang Y, Wong RJ, Stevenson DK. Unique roles of infiltrating myeloid cells in the murine uterus during early to midpregnancy. *Journal of Immunology*. 2015;194:3713-22.

Shachar BZ, Mayo JA, Lee HC, Carmichael SL, Stevenson DK, Shaw GM, et al. Effects of race/ethnicity and BMI on the association between height and risk for spontaneous preterm birth. *American journal of obstetrics and gynecology*. 2015.

Jelliffe-Pawlowski LL, Baer RJ, Blumenfeld YJ, Ryckman KK, O'Brodovich HM, Gould JB, et al. Maternal characteristics and mid-pregnancy serum biomarkers as risk factors for subtypes of preterm birth. *BJOG : an International Journal of Obstetrics and Gynaecology*. 2015

Q&A with David Relman

David Relman, MD

Professor of Medicine and of Microbiology and Immunology

Dr. David Relman is the theme leader for a project aiming to characterize the human microbiome and host response during pregnancy. This is one of the largest studies of its kind. Dr. Relman and colleagues have been collecting samples from women since the fall of 2011, gathering data regularly and frequently—weekly leading up to birth, and then monthly following birth. With approximately 105,000 samples from 275 women, the database is remarkable and still growing. The team is also conducting community-wide metagenomics to learn about the composition, dynamics, and functional potential of microbial communities, and together with collaborators at the Center, they are sampling maternal blood to explore immune function and phenotype. Upon publication of a paper in the *Proceedings of the National Academy of Sciences*, Dr. Relman spoke with us about his team's findings.

Q: *You found the microbiome remains highly stable at four body sites (vagina, distal gut, saliva, tooth/gum) during pregnancy. This seems like a surprising result. Please explain.*
A: It is quite surprising! In general, the nine or ten months of pregnancy are quite dynamic from the viewpoint of the mother's physiological state. You would think that the microbiome would be undergoing radical revision, and at least from the perspective that we've obtained so far, it doesn't seem to be the case. But, the big caveat is that the microbiome is complex. The kinds of features that we've already examined are relatively simple and focus on: Who's there? What are the species? From that perspective, it's pretty stable.

"What are the key functional features of this putative high-risk vaginal community? Is there actually a role for it in causing preterm labor? Or is it just a marker? "

Q: *What changes in women who deliver preterm?*
A: There is usually one species that dominates the vaginal community, and it's a *Lactobacillus* species, at least in Western women. But there appears to be a certain vaginal bacterial community type, which is associated with the later development of premature labor. This type of community has high diversity and tends to include large numbers of a bacterium named Gardnerella. The earlier in pregnancy this community type appeared, and the longer it persisted, the more likely that the pregnancy ended with preterm labor and delivery. The only caveat is that we know from published studies that there is also a more diverse vaginal community picture in some non-pregnant women, and they happen to be more often African American and Hispanic. That previously recognized diverse picture is somewhat similar but not identical to the picture that we are finding in the pregnancies that go on to premature labor.

Q: *And African American women are at a higher risk for delivering preterm, yes?*
A: Yes, they are at a higher risk. It begs a few simple questions that we can't answer right now, which are: What are the key functional features of this putative high-risk vaginal community? Is there actually a role for it in causing preterm labor? Or is it just a marker? It will be important for us to study more women, both pregnant and non-pregnant, and members of different races and ethnicities, in order to address these questions in a careful manner.

Another thing that we have found in most study subjects regardless of race is that there is a really sizable disturbance that takes place in the microbiome right at the time of delivery. It happens mostly in the vaginal microbial community—but also the other body sites, to some degree. And we saw it in women regardless of when they delivered (during gestation) and how they delivered (normal vaginal delivery versus cesarean delivery). The interesting thing is that this postpartum disturbance persists after delivery and it goes on for months.

Q: *What might this mean for women who want to get pregnant again?*
A: The disturbance looks like another one of these high diversity pictures characterized by a lack of *Lactobacillus* species. If you were to become pregnant at a time when you were still in the midst of this disturbance, we speculate that this might set the stage for an elevated risk for prematurity.

Gary Darmstadt, MD
Professor of Pediatrics, Neonatal and Developmental Medicine

First Person

Saving lives of preterm infants by enhancing skin barrier function

Driving from health center to health center in rural Bangladesh in 1995, I was astonished at the total absence of newborns. They were not in the hospital wards, in the lines of patients waiting to be seen in the emergency department, or in the primary care clinics. They were not even listed in the registers of patients who had visited the facilities. How could this be? When we drove to the nearby villages, I got my answer. Newborns were at home. They were born there, they received care there from village-based providers, and all-too-often they died there. They were not a priority on the global health agenda at that time. We didn't even know the number of deaths that were occurring, or what conditions caused the deaths. We lacked evidence on what worked and how to provide feasible, affordable, and effective care that could save these newborn infants.

Something that piqued my curiosity during that trip – I was a pediatric dermatology resident at Stanford at the time – was the observation that mothers, mothers-in-law and professional masseuses spent hours each day in the process of massaging newborn infants with oil, typically mustard oil. They seemed to spend more time on oil massage than any other aspect of newborn care. I came to learn that community members believed that the practice strengthened the bones, protected from cough and cold, and improved the condition of the skin. What was disturbing to me, however, was the way in which the practice was done. Mustard oil was often heated with garlic – making it even more pungent – and mixed with ground-up grains to create an abrasive concoction that could readily strip off the skin's layer of white, viscous vernix, which was perceived to be polluting for the infant. Rubbing the skin until it turned red (and thus was cleansed) and making the baby cry for a prolonged period of time were the chief indicators of a good session of oil massage that enabled the masseuse to command a high fee. (A typical oil massage session in Uttar Pradesh, India can be viewed via the link here.)

While I recognized this newborn care practice to be very important, I hypothesized that it may be adversely impacting millions of newborns each year, placing them at risk for infections and hypothermia because of damage to the skin barrier. Upon further reflection, however, I realized this could provide us with the opportunity to modify a widespread, traditional practice, introducing improvements to impact newborn health on a larger scale.

This led me first to the laboratory. I teamed up with Dr. Alfred Lane in Dermatology at Stanford, who was studying use of emollients in preterm infants in the Neonatal Intensive Care Unit, and Dr. Peter Elias at University of California, San Francisco (UCSF), a world-renowned expert in skin biology and skin barrier function. I set out to verify the impact and determine the biological basis for improved emollient therapy in preterm infants, and to apply this knowledge to low resource settings like Bangladesh. Using a mouse model of newborn skin, we found that whereas mustard oil was toxic to keratinocytes in skin and deteriorated skin barrier function, sunflower seed oil (SSO) enhanced skin barrier function. SSO is widely available and is high in essential fatty acids that can be converted to barrier-enhancing glycerols and other building blocks of the skin barrier. It is also particularly high in linoleic acid, which stimulates nuclear hormone receptors that act to accelerate skin maturation.

Armed with my findings from the laboratory, I then turned to study the impact of topical emollient therapy as a public health intervention – initially in Egypt and then in Bangladesh – for preterm infants who have a compromised skin barrier due to immaturity and undernutrition. In these low-resource settings, few options are available for management of serious bacterial infections in newborns. In a small study in a neonatal care unit in Cairo, Egypt, we showed that topical applications of SSO to the skin of preterm infants <33 weeks gestational age reduced the incidence of culture-proven

bloodstream infections by 54%. A larger study in Bangladesh showed that the intervention reduced sepsis by 41% and mortality by 26% and was highly cost effective [US$ 61 per death averted and US$ 2.15 per Year of Life Lost averted]. It appeared to act by preserving skin integrity, conserving energy by reducing loss of water and heat through the skin, and containing pathogens on the skin surface, thereby preventing invasive infections from occurring. Findings from these studies have since been replicated in a hospital-based study in Pakistan. A meta-analysis published this past year showed that topical emollient therapy reduces neonatal infections by 50% and reduces mortality in preterm infants by 25%. This intervention is now being taken back to communities where the impact on newborn mortality is being tested in large cluster-randomized trials in India and Nepal. In India, SSO is being introduced in communities in Uttar Pradesh, and they love it. Its introduction was based on very careful, formative research about how to position the therapy in the cultural and social context there, with the potential to create an enterprise model for scale-up.

Small-scale studies, including one at Stanford, have suggested that emollient therapy might also be beneficial in preterm infants in neonatal intensive care units in high income settings. However, a larger multi-center study through the Vermont Oxford Network (VON) showed no benefit in extremely preterm infants. Moreover, a subgroup analysis suggested that the therapy may put infants born at <30 weeks gestation and <500 g birthweight at increased risk for sepsis and infection. Despite these findings, I went on with clinical trials abroad because of the marked differences in context between the VON sites and the low resource settings where I work – places where oil massage is a cultural practice, where there is a much higher environmental load of highly virulent pathogens, where instrumentation (e.g., placement of indwelling lines) which can create portals for entry of pathogens that emollient therapy cannot guard against is rarely performed, and where extremely preterm infants (<28 weeks) largely do not survive. The positive results seen in Egypt, Bangladesh and Pakistan confirm that context really does matter.

Since coming back to Stanford in March of this year, I have reconnected with Dr. Elias and colleagues (Dr. Lane, a longtime mentor, has since retired from Stanford), who have succeeded in developing an optimized, inexpensive emollient (with SSO as a key ingredient) which is superior to SSO in enhancing skin barrier function in mice. We are looking for a commercial partner to manufacture the emollient, and the beneficial effects of this product are being confirmed in clinical studies in newborn infants at UCSF. Concurrently, we are preparing for clinical trials in Africa, where no data on the impact of emollient therapy in preterm infants is available. What we do know is that emollient therapy for newborns is a practice that is widespread, though highly variable, throughout Africa as in South Asia. Because it is being used on millions of newborns each year, there is broad platform for the spread of improved emollient therapy practices. We have the potential to save hundreds of thousands of lives.

I was eager to join the MOD Prematurity Research Center here. I expect to be very busy bringing the many scientific discoveries that are unfolding in laboratories across Stanford to communities around the world, impacting infants from California all the way to Kathmandu.

Contact Us

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www.prematurityresearch.org/stanford/

Spotlight: Junior Investigators

For our inaugural issue we sat down with Drs. Amy Padula and Betty Shachar to learn about their epidemiological research. Co-Principal Investigator, Dr. Gary Shaw, guides their work.

- Dr. Padula, an environmental epidemiologist, has substantially advanced our understanding over the last few years about how air pollution may be influencing reproductive health.

- Dr. Shachar, an Israeli obstetrician, recently completed her post-doctoral fellowship with our Center. Dr. Shachar is rapidly being recognized in the scientific community for her work on what short or long intervals between successive pregnancies may mean clinically.

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Q&A WITH DR. AMY PADULA

Q: *Environmental causes are associated with preterm birth, and air pollution especially is a problem in California. What are you studying?*
A: I've been interested in identifying certain air pollutants and their association with preterm birth. Most of our work is centered in the San Joaquin Valley of California where there's a lot of traffic-related air pollution. In addition to individual pollutants like carbon monoxide, nitrogen dioxide and particulate matter, we're also looking at traffic density in relation to preterm birth. Similar to other studies done at the Prematurity Research Center, we've been exploring preterm birth in categories of severity based on gestational age. Effects of air pollutants have been observed to be strongest for the earlier preterm births. We examined different periods during pregnancy to see when a critical period may be for exposure to air pollution.

Q: *You're also studying social factors. What specifically?*
A: Poverty and environmental contaminants are shown a) first of all, to be associated with one another, and b) there may be double jeopardy to being exposed both to high air pollution and poverty. We've combined data from the census on income, public assistance, and other variables that are publically available, and then investigated whether the relationship between air pollution and preterm birth differs based on your neighborhood's socioeconomic status. We found not only was the risk for preterm birth increased for mothers exposed to higher levels of each pollutant, particularly toward the end of pregnancy, but that these associations were stronger for mothers living in lower socioeconomic neighborhoods. We think that these pollutants may trigger an inflammatory response, resulting in preterm birth.

Q: *How has your research impacted environmental regulations?*
A: Over the last 40 years of standards and regulations, as science has progressed and we've found associations at lower and lower levels of air pollution, the Environmental Protection Agency continues to lower the standard of what is considered to be a healthy level of exposure.

Q: *Where do we go from here?*
A: We are thinking about investigating other environmental factors like temperature and heat waves, which are intertwined with air pollution and pesticides.

Q&A WITH DR. BETTY SHACHAR

Q: *While the World Health Organization recommends that women wait at least 24 months between a previous live birth and the conception of a new pregnancy, your recent paper written with Deirdre Lyell recommends lowering the current minimal interpregnancy interval (IPI) to 18 months. What more are you finding out now about IPI and the risk for preterm birth (PTB)?*
A: Most of the studies that have been done on this topic were done in developing countries. We decided that it would be important to look in a current US cohort to see if short IPI is still associated with the risk of PTB. We found that 30 percent of our California population [~ 1 million births from 2007-2010] had a short IPI of less than 18 months, which is a large number. A short IPI of less than six months, or less than a year, was associated with increased risk for PTB. Twelve to 17 months was at a slightly increased risk for PTB. This is consistent with a 2015 CDC report that studied 36 states and showed 30 percent of the population in the US to have an IPI of less than 18 months, which is much shorter than the WHO recommendation.

IPI is still a major issue. This is a modifiable risk factor. If it really is associated with adverse pregnancy outcomes, there is something we can potentially do about it.

Q: *Are there efforts underway to educate women about the risk associated with short IPI?*
A: Exactly. We think there is still work to do. OB/GYNs should talk and offer consultation about this association to their patients. There are family contraception programs. One of the programs is immediately after the woman delivers, to distribute contraception while she's still hospitalized. There are a lot of family programs that are trying to help, especially with groups that are at high risk for short IPI. For example, teenagers.

Q: *Where do we go from here?*
A: There are other questions that are still open and need to be investigated. For instance, what is the underlying biology associated with short IPI that is conveying the increased risk? And, for a woman with a previous pregnancy that did not result in a viable fetus or newborn, is there a high risk for a current preterm birth just because she has a short IPI? What about women with advanced maternal age? They are more interested in short IPI. We are continuing to explore these questions.