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NEWSLETTER

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PROMISING YOUNG INVESTIGATOR

MIRA MOUFARREJ



In this issue, we profile graduate student Mira Moufarrej, who works in the lab of Stephen Quake, DPhil (Theme 2 lead) and is co-first author on a paper that was published June 7 in **Science**. The paper demonstrates how non-invasive blood tests for fetal development can be used to measure gestational age with comparable accuracy to ultrasound. In parallel, it describes how blood sample analysis can be used to predict if a woman will deliver preterm. Building off of previous cell-free RNA sequencing discoveries that have come out of the Quake lab, the promising preliminary results have garnered press from **The New York Times**, **The Guardian**, and **Vox**, among other prominent publications.

Moufarrej, just 23 and two years out of graduating from MIT with a bachelor's degree, says her contribution to the study's success is a product of iterative problem solving and showing up at the right time. She began working in the Quake lab in July 2017 during her graduate rotation assignments.

The postdoc in the lab who had collected the study data had just left for a faculty position in Oregon, and Dr. Quake was looking for somebody to take on the task of analysis. Using a combination of computational and experimental biology approaches – qPCR tests, machine learning techniques, and, with luck, a data normalization strategy defined in a 1993 paper on blood tests to diagnose congenital birth defects – Moufarrej found significant results 30 minutes before one of our weekly Wednesday March of Dimes meetings.

Dr. Quake and Moufarrej rushed to alter the Power Point they were planning to share at the meeting to reflect her latest work. Arriving a few minutes late, Moufarrej remembers Dr. Quake announcing to the team: *It was really hard to analyze these spontaneous preterm birth samples. Like all of us academics do when you have a hard problem and you give it to two postdocs and two PhDs and it doesn't work, you give it to the rotation student.*

In her short time working for our Center, Moufarrej has regularly exceeded expectations. She says she thrives in defying classification, describing both her identity and her work to be “a fusion.” Moufarrej calls herself Lebanese-American; her parents are Lebanese and she was born in the US and then moved around a lot as a kid, attending middle school in Texas and high school in Dubai. She's currently a second-year PhD student in bioengineering, who has participated in classes and programs at Stanford Graduate School of Business.

“I live on this intersection where people don't understand quite what you are,” she says.

The following is excerpted from our longer interview together.

LAURA HEDLI: Compared to the other rotations that you had done as a graduate student, what about Dr. Quake's lab interested you?

MIRA MOUFARREJ: I wanted a project where I had independence to think about things on my own, something that aligned with what I found interesting. What I found in Steve's lab was good mentorship on his part on the big picture, good mentorship from the postdocs and the other lab members, and the sense of collaboration. That's really important, especially when you have someone like Steve who's busy and has so much going on. As much as the PhD is this lonely endeavor that you do on your own, I don't think that you can be a lone soldier in a problem that a lot of people are thinking about and make the strides you would hope to make.

Steve also had a collaboration with the March of Dimes and the other clinicians. I don't think I fully appreciated before starting to rotate how hard it is to get a clinical collaboration. It wasn't just that we were talking about clinical translation, it was actually there—We worked with doctors. We asked the doctors what they thought. I began to gain an appreciation for how different people think about the same problem.

HEDLI: Right, transdisciplinary team science is a hallmark of the MOD Prematurity Research Center, and this study involved team members from Stanford, University of Pennsylvania, University of Alabama, and the Statens Serum Institute in Copenhagen, Denmark. Given that you were able to draw from different perspectives throughout the data analysis process, what did you learn?

MOUFARREJ: Academics can get lost in the experimental workflow, or the simplicity of whatever you're doing without getting to an endpoint that would actually matter to a doctor. The doctors in a sense keep it real. They'll ask: Why does this matter?—Frequently.

I began to understand why a certain test would matter, what tools were at doctors' disposal, and what they did beyond what you would read in the literature. For instance, in the literature, you'll read they use ultrasound and they try to

figure out the last menstrual period. If you talk to a doctor who does it, they'll tell you it's a guessing game. They'll give you a date, but really the date's a month. Another thing we talked about was how the accessibility of good, prenatal care is dependent on when you come into the hospital, how early that is. I got a better idea of how the healthcare system funnels high-risk pregnancies.

When we think about estimating gestational age, where the current gold standard is ultrasound, this non-invasive blood test, once validated in larger studies, would be both a comparable substitute and a complement. Ultrasound's accuracy is best during the first trimester and significantly deteriorates when used during the second or third trimester. Since the accuracy of the non-invasive blood test during the second and third trimester is comparable to ultrasound's during the first trimester, we can imagine this blood test as a complementary test for gestational dating. In the case where a woman does not have access to ultrasound, like in low-resource settings, this test could be a substitute.

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HEDLI: Interestingly, your results show that the test of nine cell-free RNA transcripts for gestational age is only accurate at predicting due dates in women who deliver at term. Your other test analyzing seven cell-free RNA transcripts for preterm birth risk was able to accurately predict whether a woman was at risk for delivering preterm, but it couldn't tell you when or how early. Can you explain?

MOUFARREJ: It was interesting that even though African-American women from the second cohort were classified at-risk in general terms for preterm delivery, you could still accurately predict the time to delivery for women who delivered at full term, but not those who delivered preterm. Which seemed to

suggest to us that maybe what we were looking at and using to classify gestational age wasn't a marker of delivery per se, but was just a marker of how far along the baby was developed.

Currently, we understand very little about preterm delivery. In these pilot studies, the preterm test we developed did really well in discriminating within two high-risk cohorts, those women who delivered preterm, versus those women who were at-risk but didn't deliver preterm. These cohorts were small, however, and this work will need to be validated in larger, blinded studies. In terms of knowing the exact date of preterm delivery, we haven't figured out how to measure that yet.

HEDLI: As you continue working toward your PhD in the Quake lab, what are the experimental or computational challenges that you would like to address?

MOUFARREJ: Cell-free RNA is thought of as a diagnostic platform, which I agree with. I also think you can do cool science and look at treatment options, or open the door to things that have traditionally not been investigated in humans and don't have good animal models.

There are a lot of unanswered questions in spontaneous preterm birth that you can get at with cell-free RNA work coupled with single-cell work. If you think about cell-free RNA as a measure of what globally is going on in your body, then a) What tissues are involved? and b) What side? There are two organisms, so can we separate out signals from the mom and baby? People have tried to do this in the past, and it's hard because of both inherent limitations in what you might get out of blood and the analysis itself.

With one single RNA molecule, or one single transcript, it's really difficult to figure out what fraction is mom's and what fraction is baby's. We know that transcripts within organs correlate. Things within the placenta all seem to move in a similar fashion, and things in the immune system all seem to move in a similar fashion. If you coupled across transcripts and asked at an organ level, can we figure out how much is

mom and how much is baby? If you started seeing changes there, perhaps you could get at the potential causes of preterm birth. We could move from: "These molecules are highly predictive of preterm birth" to "These molecules are highly predictive of it and we see that they come from these tissues on this specific side." We could then couple that information with sequencing data (single-cell or bulk) from a given tissue.

The molecules we selected for gestational age, those all come from the placenta. Which makes sense. Because as the baby grows and the placenta changes, you'd see more cells dying and changeover, so you should be seeing these increases. Interestingly, the molecules that we found were predictive of preterm birth are signaling molecules that are expressed across the body. They aren't tissue-specific.

HEDLI: What do you hope to do when you finish your PhD?

MOUFARREJ: I like academia in the sense that you can explore questions that people haven't asked before, and they're very open to what you ask. It's not: "Is this going to make money?" But: "Is this important to the world?" I think that's awesome. However, I have always wanted to play a part in clinical translation, and to do that, eventually, I'd like to start my own biotech company. I chose Stanford as a PhD program specifically because I felt like I could pursue what I wanted to learn about biotech and what I wanted to learn about business in the same place as opposed to other schools where that might have not been readily accessible.

The incentives are really different between industry and what you think is cool in the lab. Really, with the backwards payer system that we have, you have to have incentives align. A business can't flourish if it's not making money, and to make money, the insurance company has to pay for it. But the insurance company doesn't want to pay. Convincing everyone in this weird ecosystem that it's a good idea takes understanding all perspectives, which you don't get just working in a lab.

MARCH OF DIMES EXPANDS TO INCLUDE INTERNATIONAL PREMATURE RESEARCH

IMPERIAL COLLEGE LONDON IS ADDED AS ITS SIXTH CENTER



**PHILLIP BENNETT,
MD, PHD**
PRINCIPAL INVESTIGATOR

The consortium of March of Dimes (MOD) Prematurity Research Centers has recently added a sixth to its ranks. The [new center at Imperial College London](#) is the first to open outside the United States and is supported by a grant from Ferring Pharmaceuticals. Director of the Institute of Reproductive and Developmental Biology at Imperial College, Dr. Phillip Bennett, is the principal investigator. Adding to an already impressive body of research on the causes and preventions of preterm birth, the Imperial College team will explore how interactions between vaginal bacteria and their maternal host may influence preterm birth.

Dr. Bennett reports MOD leadership encouraged his team to “harness the power of new strategies and new scientific approaches that haven’t yet been applied to the problem of preterm labor, and also harness the power of

people with particular expertise who might not currently be working in the preterm labor landscape.” Co-investigators of the new center include Drs. David MacIntyre and Lynne Sykes from the Institute of Reproductive and Developmental Biology at Imperial College. Leading the research efforts are Drs. Anne Dell, Ten Feizi, and Stuart Haslam, three experts in the glycosciences.

Sugar molecules, or glycans, cover the surface of every cell and enable cells to recognize one another. Indeed, Dr. MacIntyre calls it “cell handshaking.” Drs. Dell and Feizi will use complementary skills to determine which glycans are present in the cervix and vagina and what bacteria or viruses bind to them. “Whether or not the presence of a particular bacterium in the vagina leads to preterm birth will depend upon which glycans are being expressed by the mother in the vaginal epithelium, in the cervix, or in the mucus. In some cases, those glycans will allow the bacterium to be tolerated without promoting an immune response, but in other cases, different glycans might lead to the generation of an immune response,” says Dr. Bennett, summarizing the center’s overall hypothesis.

The team at Imperial College will collaborate with members at other Prematurity Research Centers, including investigators at Stanford. Stanford Principal Investigator Dr. David Stevenson says, “We look forward to coordinating our research efforts with our new partners at Imperial College and anticipate research synergy that will accelerate our quest for solutions to predict and prevent preterm birth.”

Dr. Bennett adds, “I would anticipate there would be quite a lot of interaction between us and the Stanford group in terms of the microbiome. I think we’re appreciating that actually there are quite a few technical and bioinformatics challenges to be overcome.” Even prior to the new center’s inception, Dr. MacIntyre and his colleagues had shared microbiome data with our center, which helped us validate a piece of software that can identify bacteria with a higher level of granularity.

Both centers have identified greater change in the vaginal microbiome during the first trimester of pregnancy as compared to the second or third trimesters, when the microbiome generally becomes more simple and benign. This evidence suggests we may need to look earlier when assessing risk for preterm birth and miscarriage. To this end, data exchange among our centers may be highly advantageous, especially if we observe geographical and ethnic differences between women in the U.S. and Europe.

Imperial College London is situated in a part of the city with high ethnic diversity and an influx of new immigrants. The center will bring together researchers at Imperial College with personnel, patients, and resources at three major London hospitals: Queen Charlotte’s Hospital, St. Mary’s Hospital, and Chelsea and Westminster Hospital.

Establishing the first partnership between academia and industry in the family of prematurity research centers, Ferring Pharmaceuticals is providing funding support for the new center. Headquartered in Saint-Prex, Switzerland, Ferring has a strong interest in reproductive medicine and women’s health, and one-third of its research and development investment goes toward finding healthcare solutions for mothers and babies. “A University is not very good at turning a discovery into a product,” Dr. Bennett says. “I’ve always felt there should be close collaboration between University groups and the pharmaceutical industry.”

RECENT PUBLICATIONS, A SAMPLING

IN 2018, WE’VE HAD 14 PUBLISHED ARTICLES TO DATE

Ozen M, Zhao H, Kalish F, Yang Y, Folkins A, Burd I, Wong RJ, Stevenson DK. [Heme oxygenase-1 deficiency results in splenic T-cell dysregulation in offspring of mothers exposed to late gestational inflammation](#). *Am J Reprod Immunol*. 2018 May;79(5):e12829.

Ngo TTM*, Moufarrej MN*, Rasmussen MH, Camunas-Soler J, Pan W, Okamoto J, Neff NF, Liu K, Wong RJ, Downes K, Tibshirani R, Shaw GM, Skotte L, Stevenson DK, Biggio JR, Elovitz MA, Melbye M**, Quake SR** (*=joint first authors, **=joint corresponding authors). [Noninvasive blood tests for fetal development predict gestational age and preterm delivery](#). *Science*. 2018 Jun 8;360(6393):1133-1136.

Li J, Hong X, Mesiano S, Muglia LJ, Wang X, Snyder M, Stevenson DK*, Shaw GM.* [Natural selection has differentiated the progesterone receptor among human populations](#). *Am J Hum Genet*. 2018 Jul 5;103(1):45-57.

Shaw GM, Yang W, Roberts EM, Aghaeepour N, Mayo JA, Weber KA, Maric I, Carmichael SL, Winn VD, Stevenson DK, English PB. [Residential agricultural pesticide exposures and risks of preeclampsia](#). *Environ Res*. 2018 Jul;164:546-555.
