MICRORNA PROFILES AND PSYCHOSOCIAL STRESS DURING PREGNANCY IN A COHORT OF URBAN HISPANIC MOTHERS

HELEN BERMUDEZ FOLEY, PHD

DIVISION ENVIRONMENTAL HEALTH

DEPT PREVENTIVE MEDICINE

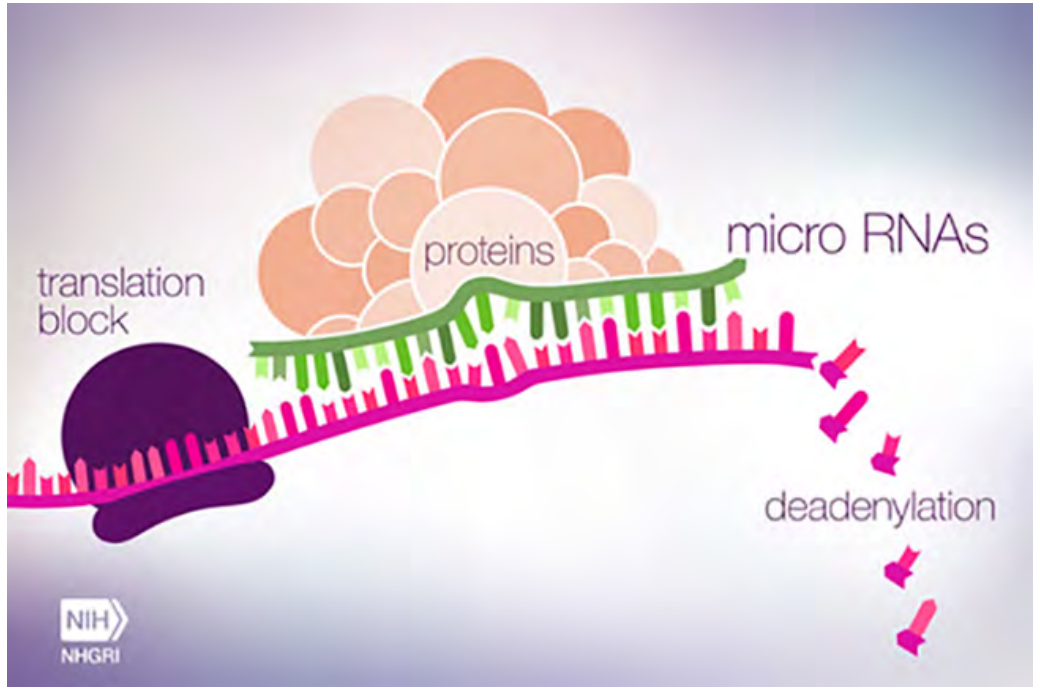
USC

OUTLINE

- miRNAs and pregnancy
- The MADRES cohort
- Studying miRNA and health
- Results miRNA and Psychosocial stress
- Remaining analysis and conclusions

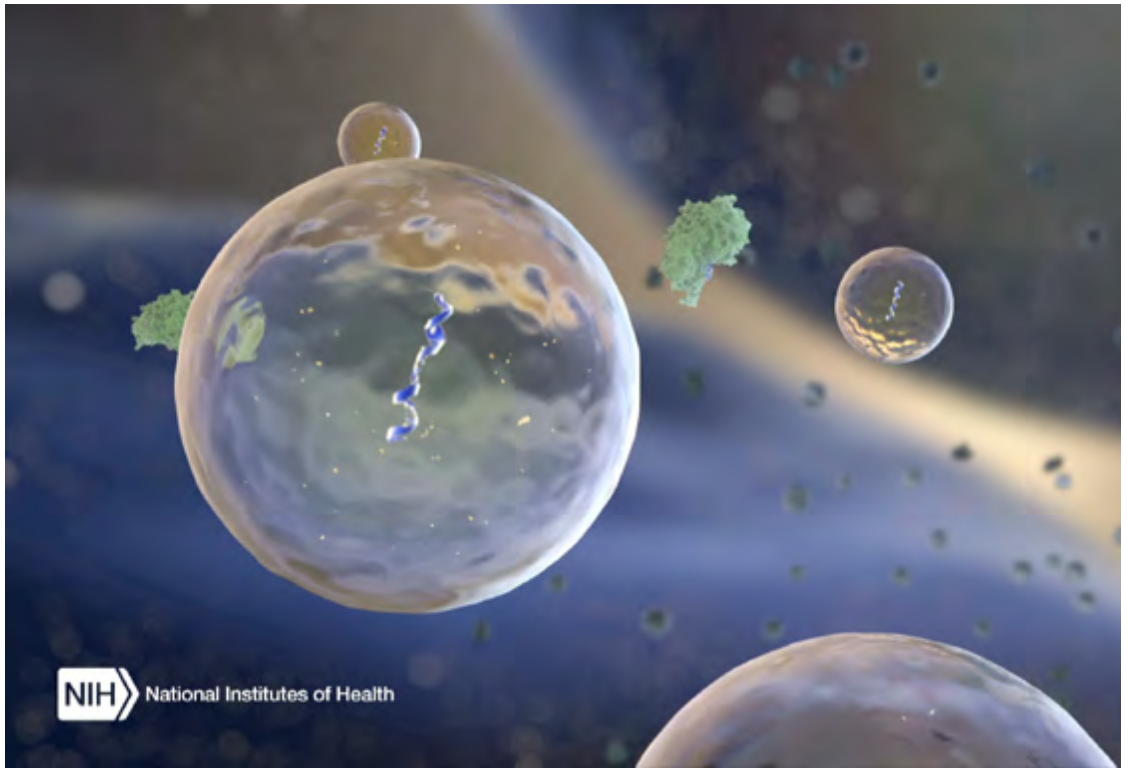
MIRNA CHANGES WITH ENVIRONMENT AND HEALTH

- miRNA are ~22nt segments of RNA that modulate gene expression.
  - More than 2000 in humans!
- Sensitive to environment and conditions- Biomarker potential (Gilad, 2008)
- Change in biofluids:
  - Cancer (Ogata-Kawata, 2014; Armstrong, 2015)
  - Diabetes (Garcia-Contreras, 2017)
  - Alzheimer's Disease (Lugli, 2015)



EXOSOMAL MIRNA IS IMPORTANT DURING PREGNANCY

- Exosomes are extracellular vesicles (~40-100nm) Carrying miRNA, RNA, proteins, lipids etc.
  - Intracellular communication (Adam, 2017)
- Particularly interesting: placental in origin and circulating in the mother's plasma (Luo, 2009)
- During pregnancy, changes in miRNA are associated with:
  - Preeclampsia (Ospina-Prieto, 2016)
  - Preterm birth (Fallen, 2018)
  - Gestational Diabetes (Cao, 2017)
  - But are also part of a normal pregnancy (Ospina-Prieto, 2013)

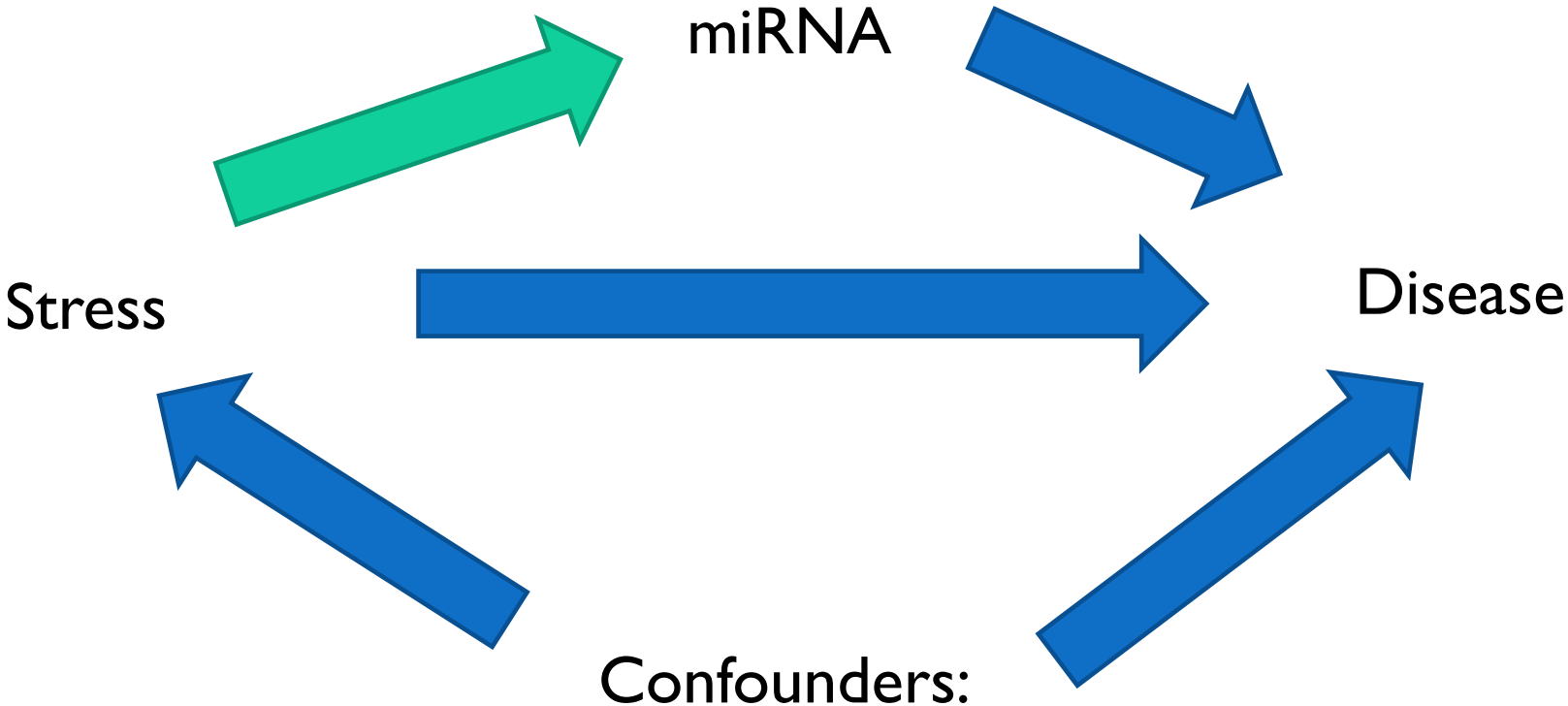


EPIDEMIOLOGY

- "…the study of the distribution and determinants of health-related states or events" (World Health Organization)
  - Who? When? Where?
- Pregnancy Outcomes (Kramer, 2003)
  - Term and preterm birth
  - Neonatal height, weight, etc.
  - Infant mortality
  - Effects from environment: Lead, pollution, nutrition, etc.
- Developmental Origins of Health and Disease (DOHaD)

Oct 2010

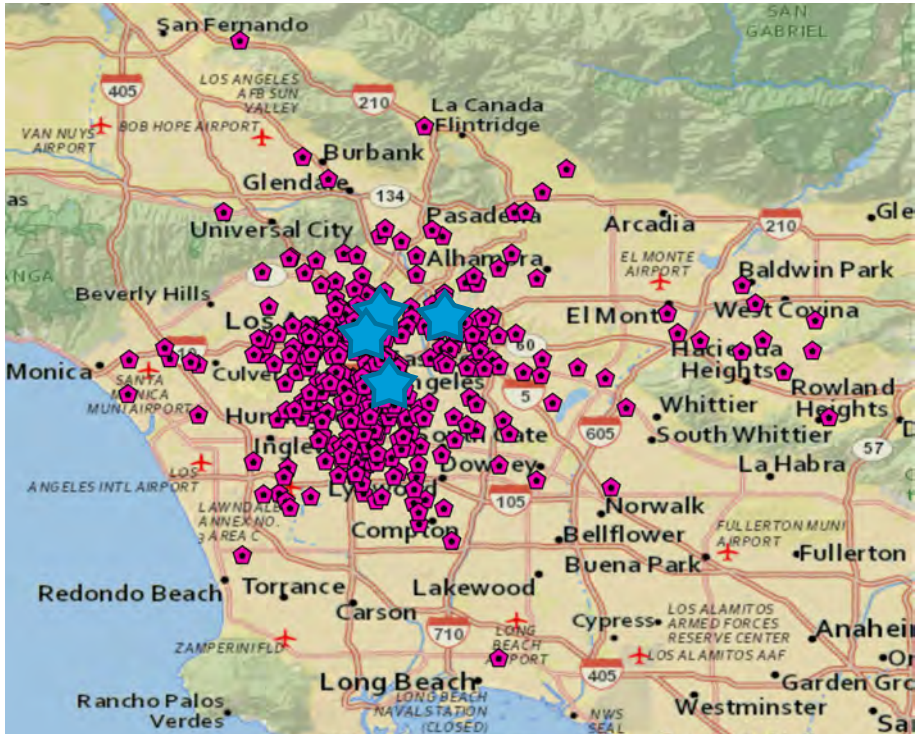
MIRNA CHANGES DUE TO STRESS



MADRES COHORT

To understand causes underlying health disparities in childhood obesity and excessive weight gain and postpartum weight retention among minority and low-income women in Los Angeles

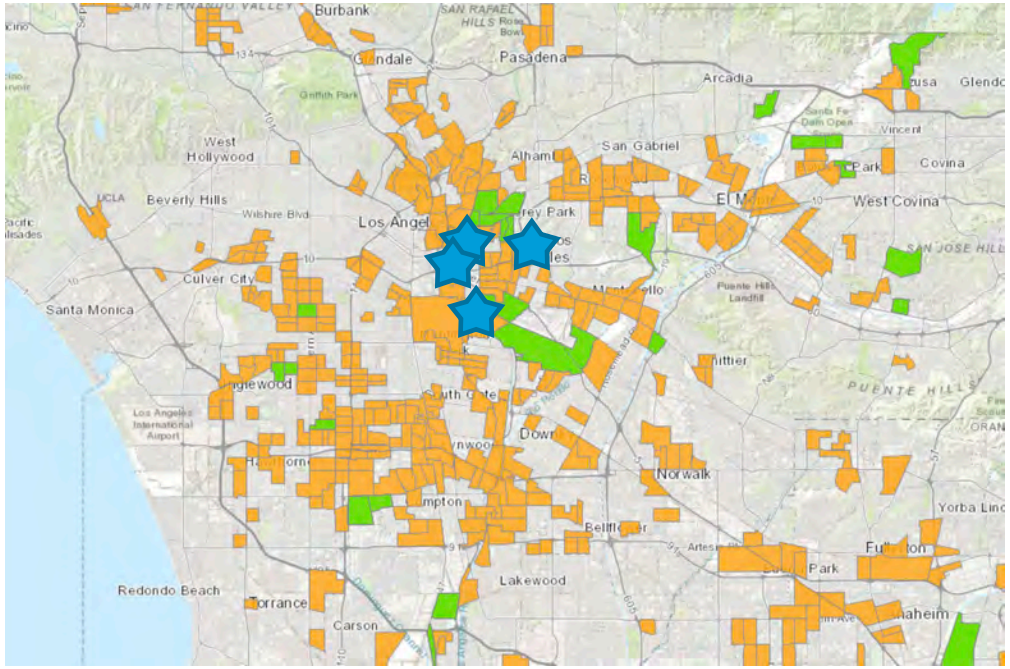
- Predominantly low-income Hispanic women
  - Projected n=750, current n=680 with 467 births
  - ~75% have household income <$30k
- Recruited before 30 weeks, but as early as 5 or 6!
- Several visits during pregnancy, with follow-up through baby's first year of life.
  - Trimester 1 (6-12 weeks)
  - Trimester 3 (28-40 weeks)



MADRES COHORT

To understand causes underlying health disparities in childhood obesity and excessive weight gain and postpartum weight retention among minority and low-income women in Los Angeles

- Predominantly low-income Hispanic women
  - ~37% have ≥ 5 people in the household
  - ~27% did not complete high school
  - ~91% using Medi-Cal insurance
- Many environmental health risk factors
  - Proximity to freeways and industry
  - Food deserts
- But also (sometimes) high social support
  - Neighborhood cohesion
  - Emotional and social support resources



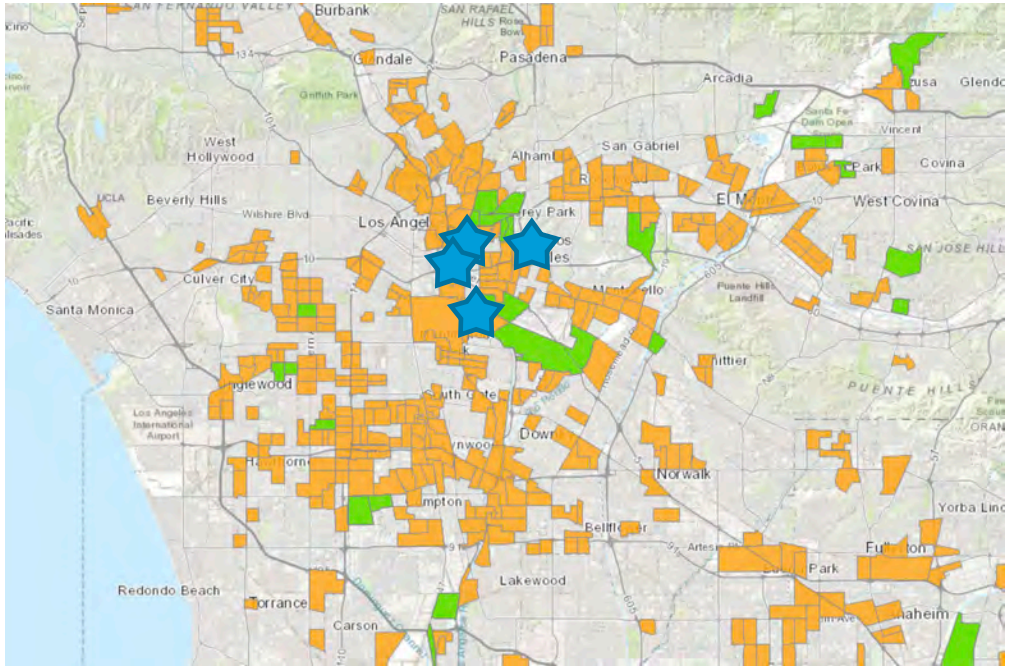
Food desert map, (USDA, 2015)

MADRES COHORT

To understand causes underlying health disparities in childhood obesity and excessive weight gain and postpartum weight retention among minority and low-income women in Los Angeles

Wealth of data on these participants:

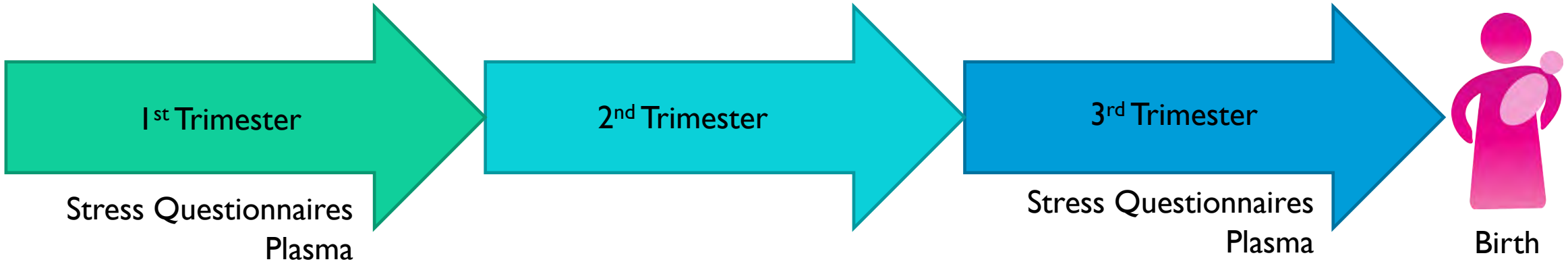
- Repeated measures over pregnancy
- Questionnaires on demographics, history, social stress, depression, etc..
- Addresses (and retrospective addresses)
- EMR data
- Biospecimens: blood, urine, hair, nails, feces, saliva
- Different assays: DNA methylation, metals exposure, cortisol & biomarkers

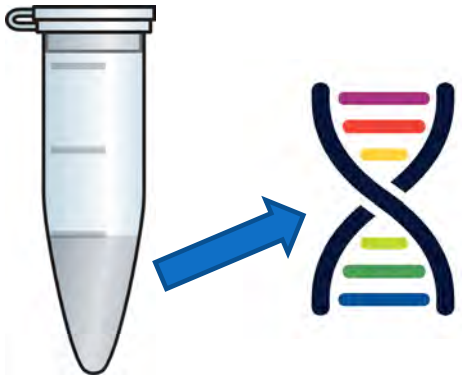


Food desert map, (USDA, 2015)

STUDY DESIGN

Target n=500 samples from first and third trimester





- Extract exosomal miRNA
- Quantify with Nanostring (~800 miRNA)
- Link by participant ID to demographic data and stress questionnaires
- So far: 200 samples from 147 participants
  - Subset for these results

MIRNA COHORT CHARACTERISTICS

	Mean (SD) or N(%)*			N (%)*
Maternal Age (n=147)	29.52 (5.70)		**Marital Status**	
			Married	38 (25.9%)
			Living together	64 (43.5%)
			Single	25 (17.0%)
			Divorced	5 (3.4%)
Gestational Age			**Language**	
First Trimester (n=77)	13.50 (4.03)		English	80 (54.4%)
Third Trimester (n=70)	31.61 (2.09)		Spanish	53 (36.1%)
Income			**Race**	
<15k	37 (25.2%)		Non-Hispanic Black	14 (9.5%)
15-29k	42 (28.6%)		Non-Hispanic Other	15 (10.2%)
30-49k	17 (11.6%)		Other Hispanic	12 (8.2%)
50-99k	5 (3.4%)		White Hispanic (US-born)	41 (27.9%)
100k or more	10 (6.8%)		White Hispanic (Foreign-born)	61 (41.5%)
Don't Know	23 (15.6%)		Unknown	4 (2.7%)

*Percentages may not sum to 100% if participants chose not to answer.

MODEL WITH STRESS

- Linear Model: miRNA ~ Stress + Confounders
- Stress as measured by questionnaire instruments at both trimesters with miRNA data
  - Perceived Stress Scale (PSS)
  - Prenatal Distress Questionnaire (PDQ)
  - Center for Epidemiologic Studies – Depression (CES-D)
- Confounders known (or suspected) to affect miRNA expression in pregnancy
  - Gestational Age
  - Maternal Age
  - Additional control for recruitment site
- For each of 73 miRNA candidates

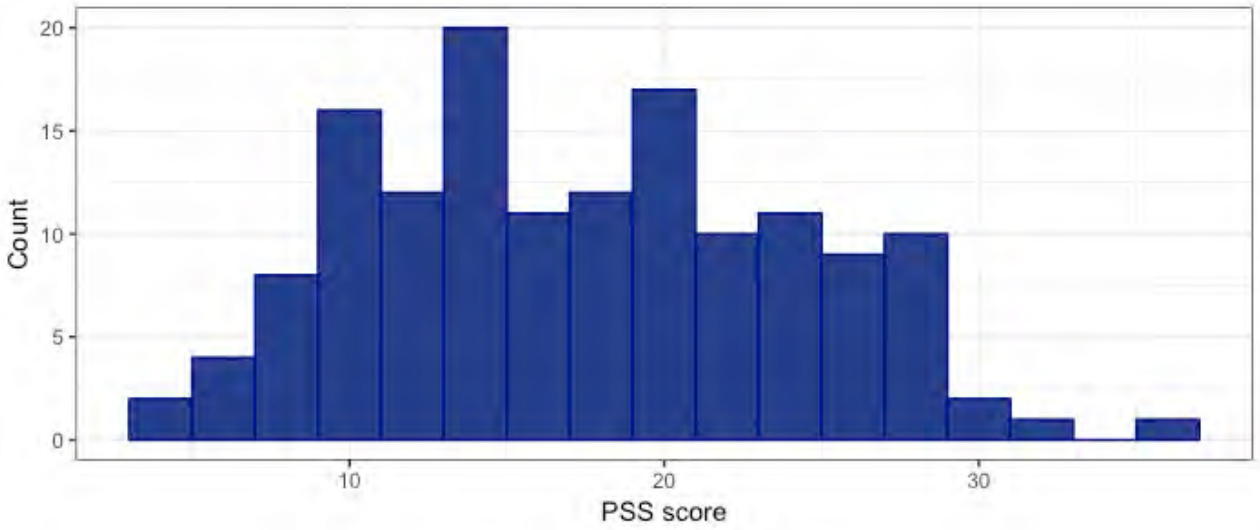
PERCEIVED STRESS SCALE (PSS)

- 10 Questions answered on a scale of 0-4, reflecting on the past 30 days.
  - Range: 0-40
- Evaluated at T1 and T3
  - linked to plasma sample
- Our participants:
  - Min: 4
  - Max: 37

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4

Cohen, 1983



MIRNA CHANGING DUE TO STRESS

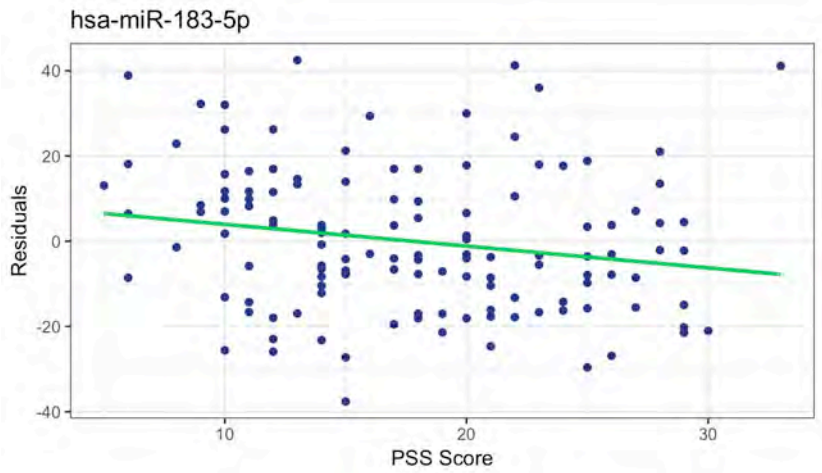
73 models, one for each miRNA: *miRNA ~ PSS + (Gestational Age, Maternal age, Recruitment Site, Race)*

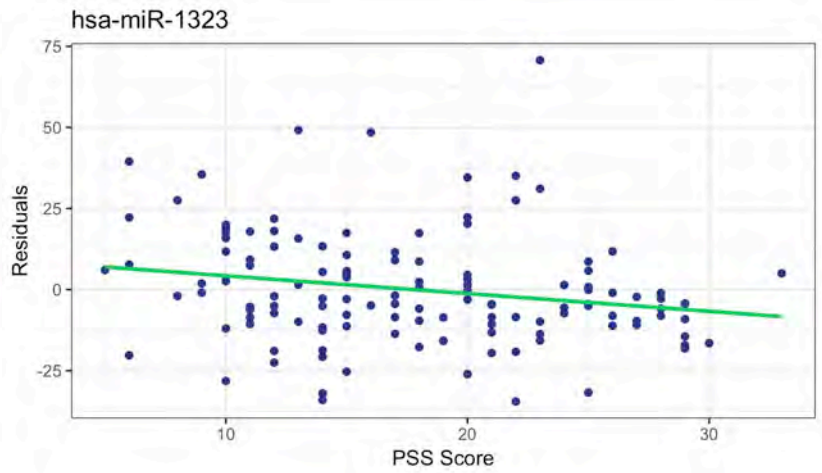
miRNA Name	PSS Estimate (CI)	p-value
hsa-miR-1252-5p	-1.38 (-2.22, -0.544)	0.00143
hsa-miR-548y	-0.723 (-1.16, -0.284)	0.00146
hsa-miR-1323	-0.569 (-1.05, -0.0887)	0.0207
hsa-miR-329-3p	-0.925 (-1.72, -0.127)	0.0234
hsa-miR-1295a	-0.517 (-0.972, -0.0622)	0.0262
hsa-miR-183-5p	-0.531 (-0.999, -0.0627)	0.0266
hsa-miR-4516	0.921 (0.106, 1.74)	0.0271
hsa-miR-1255a	-0.47 (-0.921, -0.0197)	0.0409

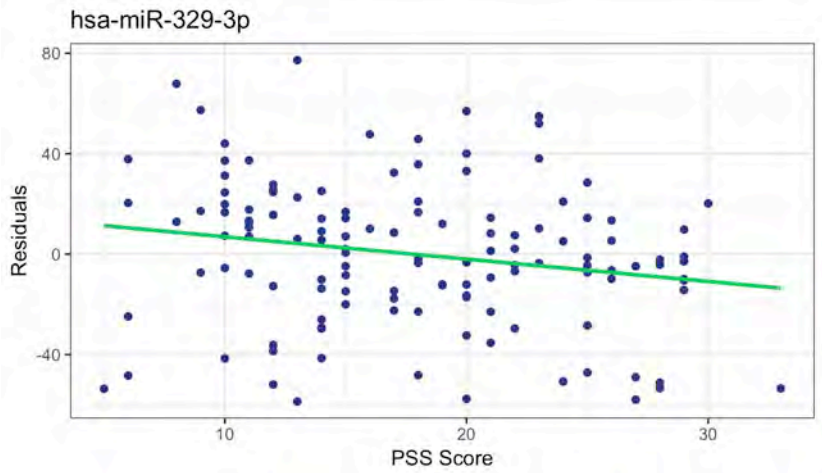
SUBTLE EFFECTS FROM STRESS

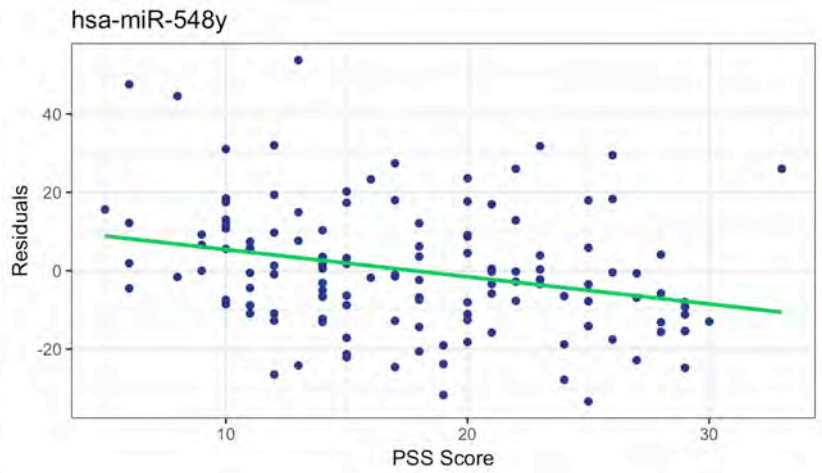
"Essentially, all models are wrong, but some are useful."
-George Box

What else can we look for?





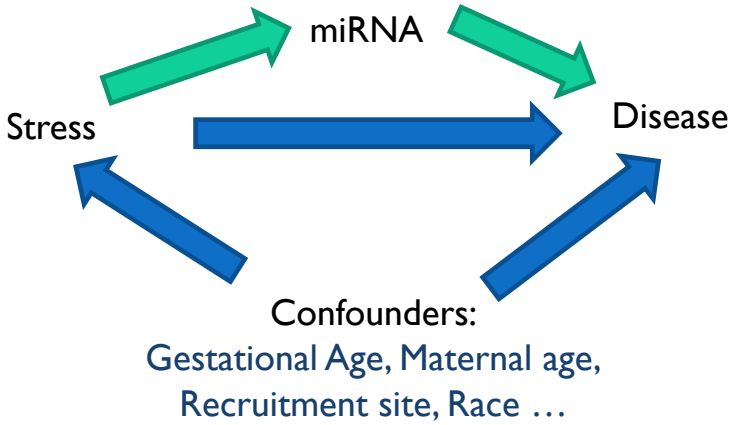




POLYGENIC EFFECTS

Each of the miRNA has many predicted targets, making it difficult to pinpoint the mechanisms at play.

But oncogenic and anti-apoptotic miRNA targets dominate the first trimester, while cell differentiation targets are prevalent in third trimester. (Gu, 2013)



miRNA Name	Number of predicted targets (miRDB.org)	Top target characteristics
hsa-miR-1252-5p	1262	Immune expression: lymph nodes, spleen
hsa-miR-548y	1594	Regulates tumor suppression
hsa-miR-1323	1145	Protein trafficking
hsa-miR-329-3p	584	Apoptosis regulation
hsa-miR-1295a	36	Cell-proliferation and/or differentiation?
hsa-miR-183-5p	565	Actin binding in response to extracellular signals
hsa-miR-4516	1586	Spermatogenesis?
hsa-miR-1255a	369	Placenta-specific expression

FUTURE DIRECTIONS

- Ongoing recruitment and data collection
  - N=500 1st and 3rd trimester
  - Sensitivity and power
  - Repeated measures for miRNA change
- Address other confounders in models
  - Other health-related factors: antibiotic usage, GDM, BMI …
  - Stratified analysis for trimesters, fetal sex, and race
  - Logistic regression for low-expression miRNA
- miRNA associations with other outcomes
  - Birth weight and length
  - Diabetes, hypertension, pre-eclampsia

ACKNOWLEDGEMENTS

Investigators

- Carrie Breton
- Frank Gilliland
- Tracy Bastain
- Rima Habre
- Genevieve Dunton
- Claudia Toledo-Corral
- Shohreh Farzan
- Brendan Grubbs
- Jill Johnston

Postdocs, Students, Analysts

- Caitlin Howe
- Mariam Girguis
- Thomas Chavez
- Tahlia Hodes
- Christine Naya
- Daniel Chu
- Ashley Song
- Alicia Peterson

Laboratory staff

- Carmela Guerrero
- Lili Gevorkian
- Eileen Granada
- Shanyan Xue

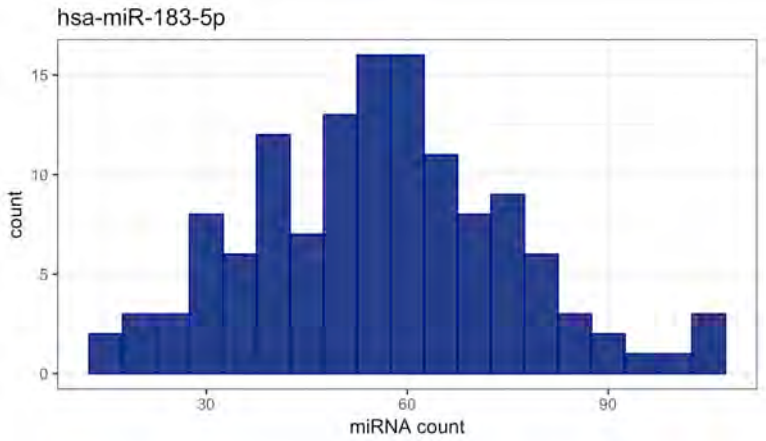
Community Engagement

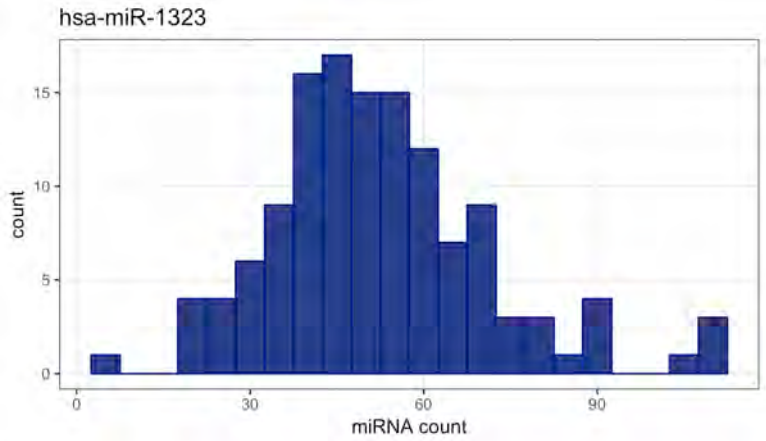
- Wendy Gutschow
- Zully Juarez

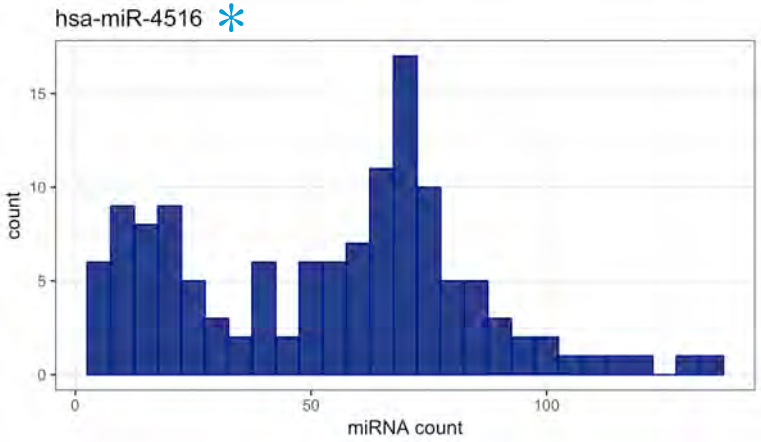
Data collection staff

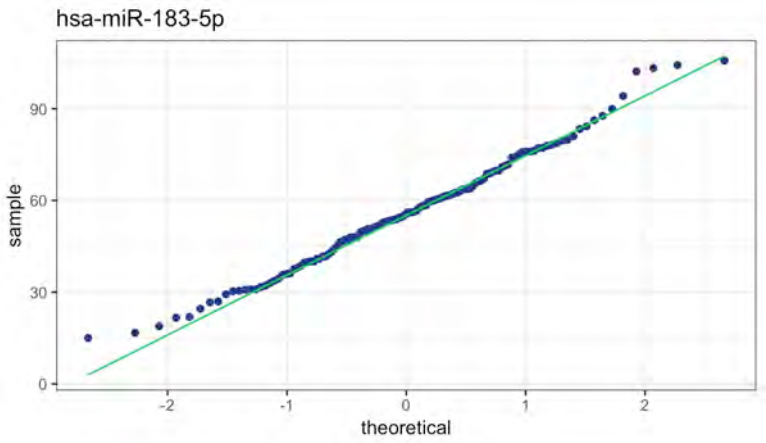
- Milena Amadeus
- Jane Cabison
- Jackie Garcia-Enriquez
- Jessica Medel
- Ana Roman-Calixto
- Carmen Chavez
- Marisela Miranda
- Andrea Calderon
- Dema Fahem
- Lisa Valencia
- Marisela Rosales

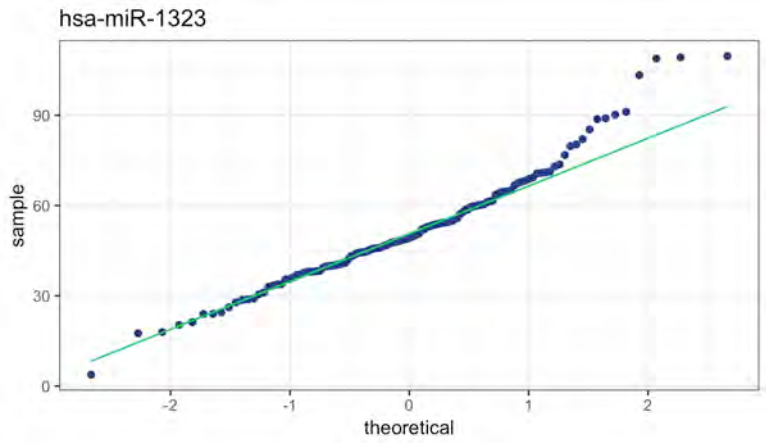
NORMALITY

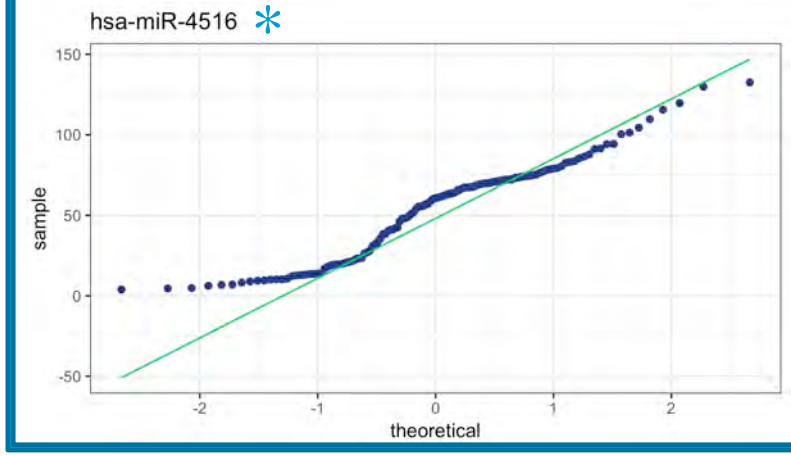

hsa-miR-183-5p
count
miRNA count


hsa-miR-1323
count
miRNA count


hsa-miR-4516 *
count
miRNA count


hsa-miR-183-5p
sample
theoretical


hsa-miR-1323
sample
theoretical

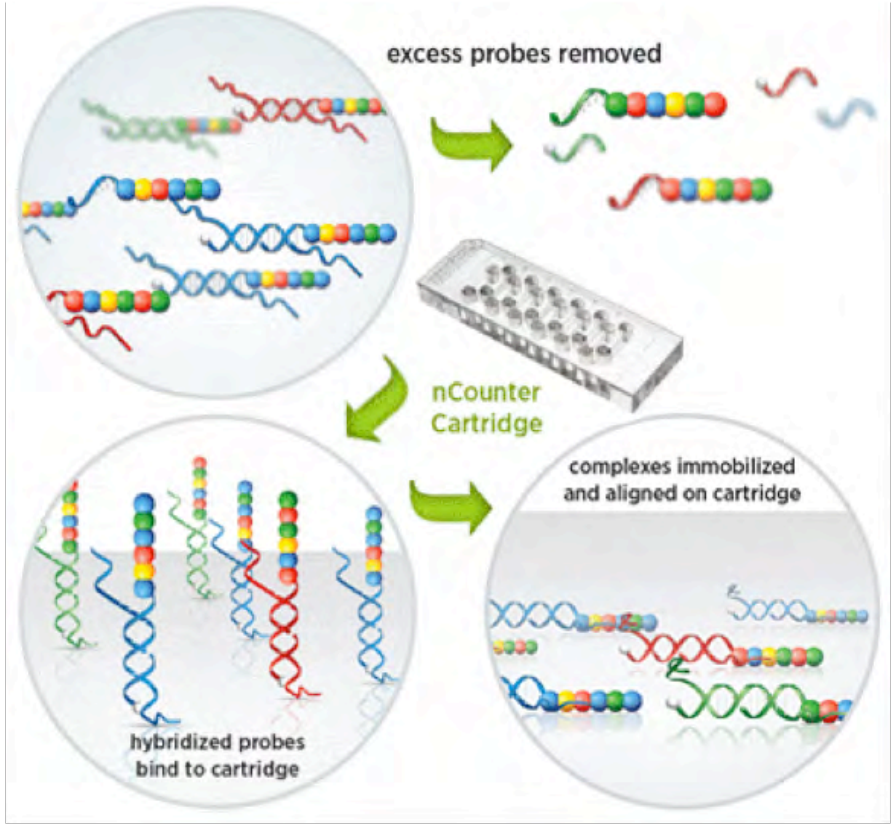

hsa-miR-4516 *
sample
theoretical

MIRNA AND ANTIBIOTIC USAGE

- miRNAs have MANY other effects
  - Modulates immune system,
  - Bacterial infection (Zhou, 2018)
  - Viral infection (Roberts, 2011)
  - Immune system effects in pregnancy (Bidarimath, 2014)
- Of the 10 outliers removed due to high miRNA content, 8 of them were diagnosed (and treated for) some infection (UTI, STD, Respiratory infection)
- Add short-term stressors to the model and address variation in miRNA responses.
  - Consider other effects of immune modulation and miRNA in pregnancy
  - Immune components of pregnancy complications

NANOSTRING DATA

- Clever fluorescent hybridization
- Profile for ~800 human miRNA, plus these (for each sample):
  - 8 negative controls
  - 6 positive controls
  - 5 spike ins (lab added 2, cel-254, osa-442)
  - 5 housekeeping miRNA
  - 6 ligation controls



DEMOGRAPHICS OF MIRNA PROFILED GROUP

- 147 participants (total of 200 timepoints) – 4 have no race/ethnicity demographic data and were dropped
- Race (White, Black, Asian, Native American, etc.) and Hispanic (Y/N) data on n=143 (196 timepoints)
  - 153 White Hispanic
  - 20 Black Non-Hispanic
  - 19 Non-Hispanic (White, Asian, other)
  - 7 Other Hispanic (mixed, black, other)
- Among White Hispanics, 90 are foreign-born and 57 are US-born. (13 declined to state.)

MIRNA : PSS BETAS

73 models, one for each miRNA: *miRNA ~ PSS + (Gestational Age, Maternal age, Recruitment Site, Race)*

miRNA Name	Estimate (CI)	p-value	FDR-adj p-value
hsa-miR-1252-5p	-1.38 (-2.22, -0.544)	0.00143	0.0532
hsa-miR-548y	-0.723 (-1.16, -0.284)	0.00146	0.0532
hsa-miR-1323	-0.569 (-1.05, -0.0887)	0.0207	0.283
hsa-miR-329-3p	-0.925 (-1.72, -0.127)	0.0234	0.283
hsa-miR-1295a	-0.517 (-0.972, -0.0622)	0.0262	0.283
hsa-miR-183-5p	-0.531 (-0.999, -0.0627)	0.0266	0.283
hsa-miR-4516	0.921 (0.106, 1.74)	0.0271	0.283
hsa-miR-1255a	-0.47 (-0.921, -0.0197)	0.0409	0.373