# Genetic epidemiology of early growth-cardiometabolic disease links

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# Prior to joining NICHD…

## 2004– Podoconiosis: public health to genetics, back to public health

- **Socio-economic burden**

Tekola-Ayele et al. *Trop Med* 2006
Tekola-Ayele et al. *PLoS Neg Tr Dis* 2009
Tekola-Ayele et al. *BMC Med Eth* 2010

- **Develop clinical grading system**


Tekola-Ayele et al. *Trop Med* 2008

- **Genetics**

The NEW ENGLAND JOURNAL of MEDICINE

HLA Class II Locus and Susceptibility to Podoconiosis

Tekola-Ayele et al. *NEJM* 2012

- **Public health translation**

World Health Organization

Neglected tropical diseases

**Podoconiosis: endemic non-filarial elephantiasis**

Podoconiosis is a type of tropical lymphoedema clinically distinguished from lymphatic filariasis (LF) through being ascending and commonly bilateral but asymmetric. Evidence suggests that podoconiosis is the result of a genetically determined abnormal inflammatory reaction to mineral particles in irritant red clay soils derived from volcanic deposits.

Tekola-Ayele et al.
*J Comm Genetics* 2015

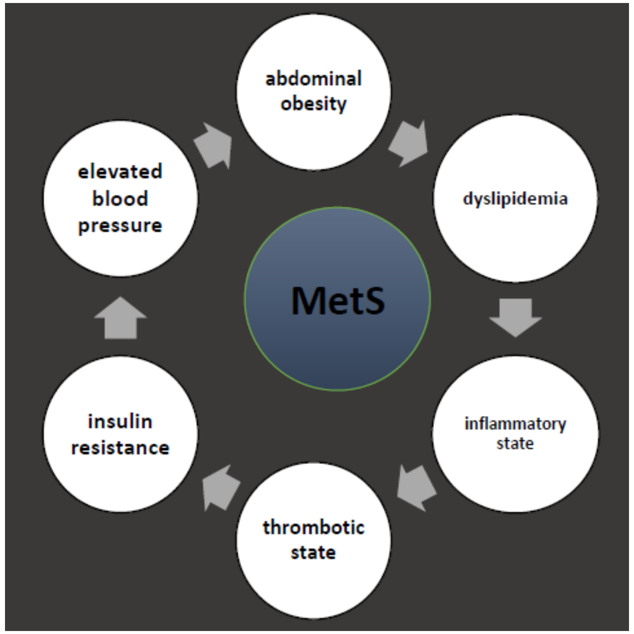
# 2011–2016 genetics of cardiometabolic diseases, population genetics

- **African Genome Variation Project**


Gurdasini*, Tekola-Ayele* et al. *Nature* 2015

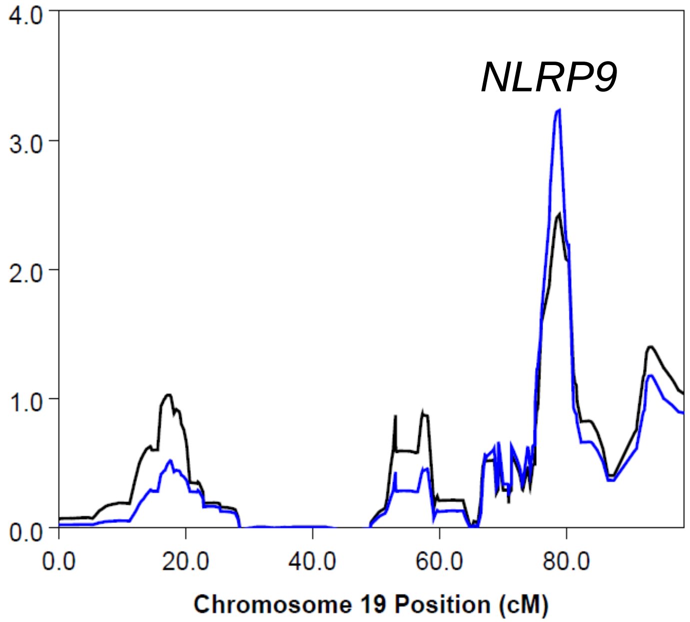
*co-first author

- **Metabolic syndrome**




Tekola-Ayele et al. *Mol Gen Met* 2015

- **Type 2 diabetes**



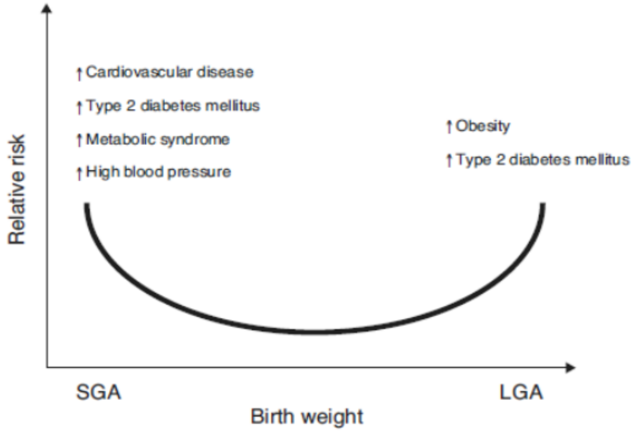

Tekola-Ayele et al. *Pharmacogenomics J* 2014

# NICHD 2016-

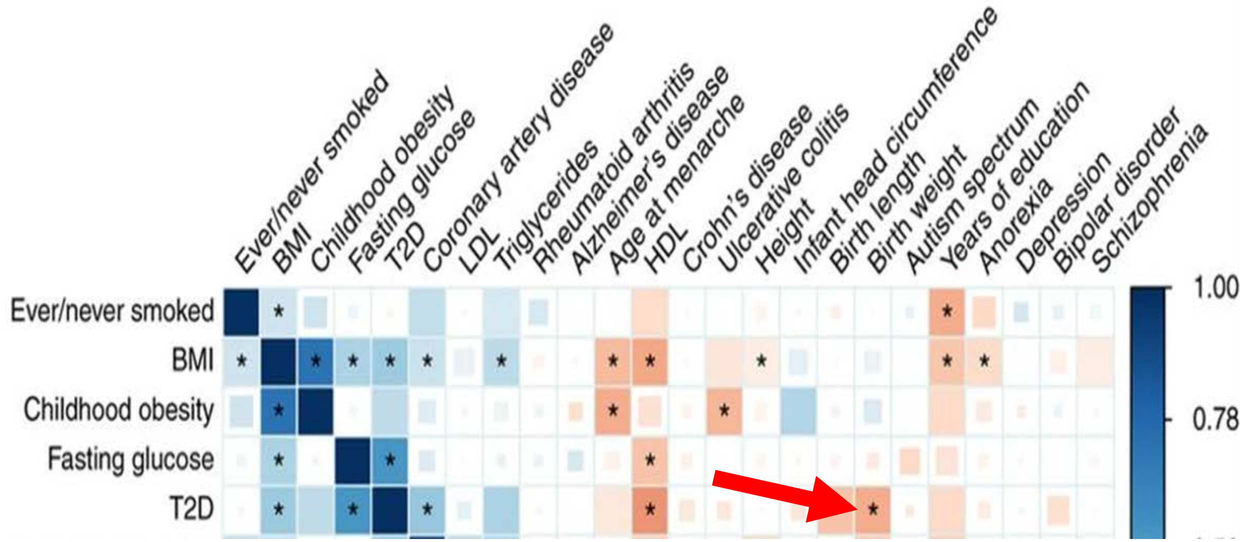
## Genetic-epidemiology of early growth-cardiometabolic diseases

### Motivation

The early life period is one of the critical times in health across the life course



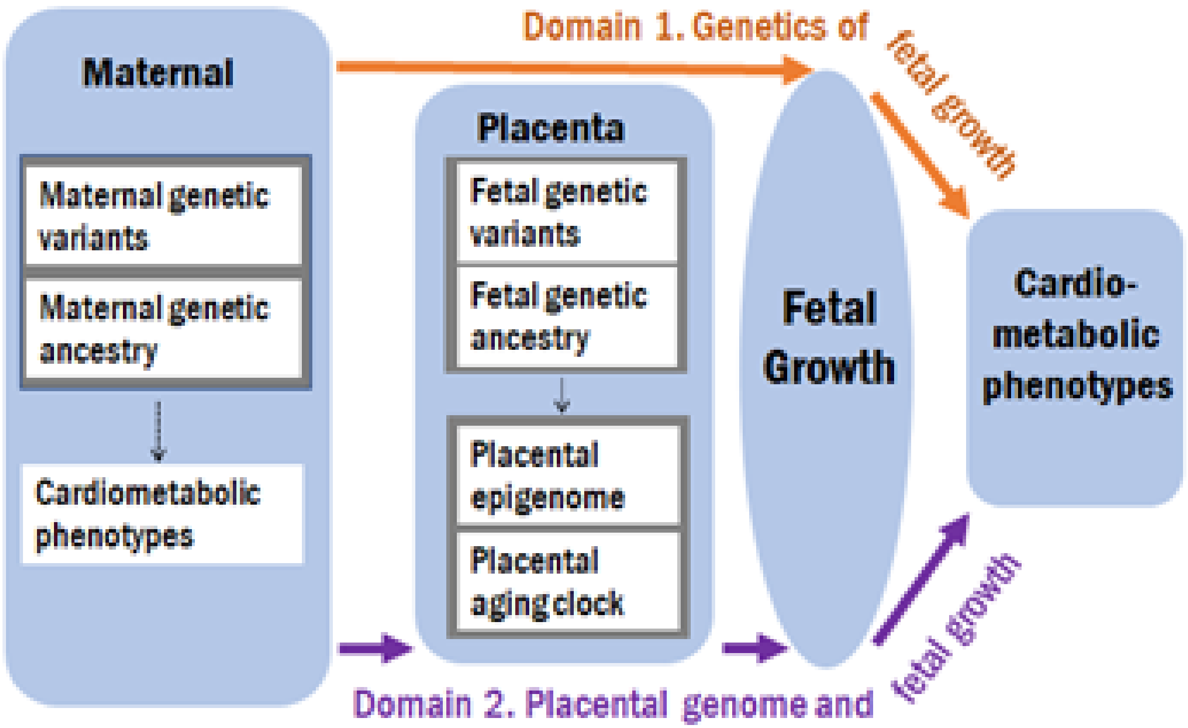

Ramirez-Velez *Endocrinol Nutr.* 2012; 59:383-93;
Barker et al. *Ped Perinat Epi* 1992




Bulik-Sullivan et al. *Nature Genetics* 2015

# Research program

**<u>Goal</u>: genetic mechanisms of early growth variations and links with cardiometabolic outcomes.**




- **Genetic regulation of fetal growth**

  Tekola-Ayele et al. *PLoS Genetics* 2020
  Rahman ... Tekola-Ayele *JCEM* 2019
  Tekola-Ayele et al. *Hum Genomics* 2019
  Tekola-Ayele et al. *Scient Reports* 2019
  Ouidir ... Tekola-Ayele *J Clin Lipid* 2019
  Tekola-Ayele et al. *BMC Medicine* 2018
  Shrestha … Tekola-Ayele *Front Genetics* 2018
  Shrestha … Tekola-Ayele *Obesity* 2018
  Workalemahu … Tekola-Ayele *Scient Reports* 2017

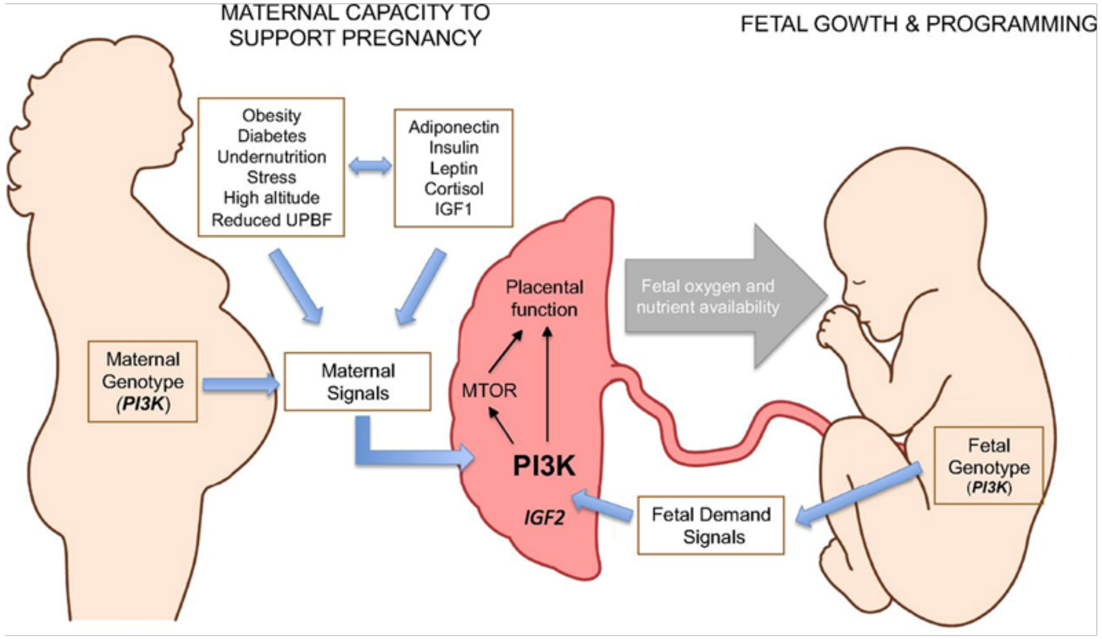
- **Placental genome/aging & fetal growth**

  Tekola-Ayele et al. *Clinical Epigenetics* 2020
  Ouidir … Tekola-Ayele *Epigenomics* 2020
  Tekola-Ayele et al. *Aging* 2019
  Workalemahu … Tekola-Ayele *Hypertension* 2019
  Shrestha …Tekola-Ayele *Int J Obesity* 2019
  Workalemahu … Tekola-Ayele *J Dev orig Health Dis* 2019
  Shrestha … Tekola-Ayele *Epigenetics* 2018

# Placenta, aging & fetal growth

- The placenta supports pregnancy and undergoes physiologic aging
- Some placentas may show signs of accelerated aging
- Disrupted aging of placenta – based on pathologic & telomerase markers– may lead to pregnancy complications

(Behnia et al. *Placenta* 2015, 36: 969–973)




*PNAS* 2016; 113: 11066-68

# Measuring aging “clock” using epigenetic markers

- Accelerated aging leads to functional decline but measuring age acceleration is challenging

- Epigenetic clock is a promising molecular estimator of biological age
  - Epigenetic age predicts chronological age with high accuracy
  - Age acceleration = epigenetic age – chronological age
  - High heritability
  - Predicts cancer, cardiovascular diseases, mortality in adults
  - Early onset preeclampsia

(Horvath & Raj *Nature Rev Genet* 2018, 19:371-84; Horvath *Genome Biol* 2013,14:R115; Behnia et al. *Placenta* 2015, 36: 969–973)

# Placental epigenetic aging studies

- Genetic susceptibility, ancestry
- Relations with fetal growth, sex differences
- Maternal factors (e.g., cardiometabolic, psychosocial)
- Molecular biomarkers of placental aging

- Age acceleration can have consequences on fetal growth
- Male fetuses more vulnerable to adverse neonatal outcomes, severe placental histopathological lesions
- Sex differences in placental response to adverse perinatal exposures, and epigenomic/transcriptomic profiles

(Naeye et al. *Pediatrics* 1971, 902–06)

# Hypothesis

**Sex-specific associations of placental age acceleration with fetal growth, neonatal anthropometry measures, and risk of low birth weight.**

Fetal Growth Study
NICHD

- The NICHD Fetal Growth Studies – Singletons
  - a prospective cohort of 2,802 pregnant woman
- Gestational age confirmed by ultrasound
- Fetal growth measured by ultrasound at 5 gestation times & standard neonatal anthropometry
- 301 women provided placental samples at delivery

(Buck Louis et al. *Am J Obstet Gynecol* 2015, 213:449.e1;Grewal et al. *Int J Epidemiol* 2018, 47:25)

# Placental and maternal DNA profiling

**Placenta**
**(n=301 after QC)**
(biopsies in RNALater: 0.5cm x 0.5cm x 0.5cm, below fetal surface, within 1 hr of delivery)

- Methylation: Infinium Human Methylation450 Beadchip → Epigenetic age (62 CpGs)
- Genotyping: HumanOmni2.5 Beadchip → Fetal genetic ancestry

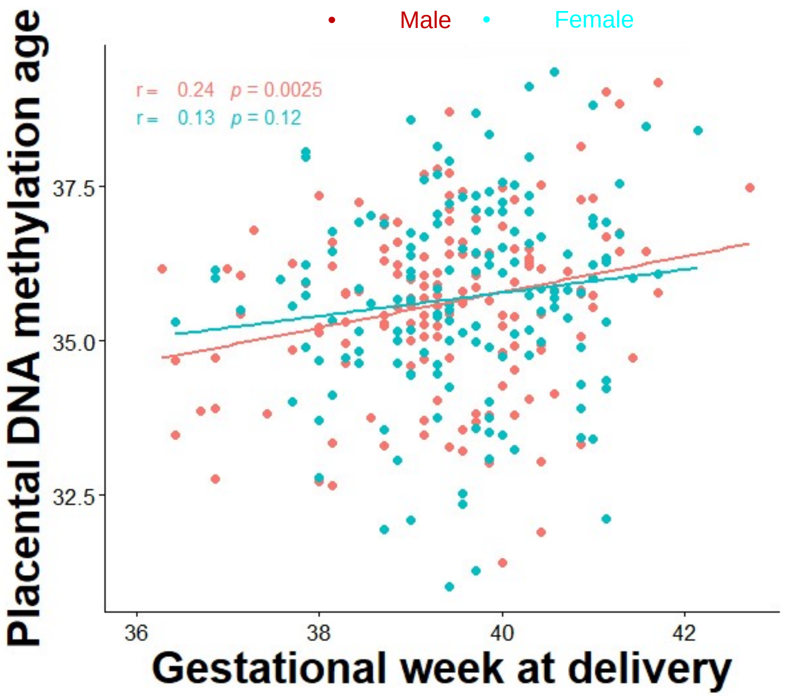
**Maternal DNA**
**(n=2065)**

- → Genotyping: MultiEthnic Genotyping Array (n=2650) → Maternal genetic ancestry

**Age Acceleration** = DNA methylation age – gestational age

# Characteristics of study participants

| | Mean ± SD or n (%) | |
|---|---|---|
| | Female offspring (n=149) | Male offspring (n=152) |
| Maternal age, n (%) | | |
| <30 yrs | 89 (59.7) | 93 (61.2) |
| 30-35 yrs | 44 (29.5) | 45 (29.6) |
| ≥35 yrs | 16 (10.7) | 14 (9.2) |
| Gestational age at delivery, wk | 39.6 ± 1.1 | 39.4 ± 1.2 |
| Race/ethnicity, n (%) | | |
| White | 38 (25.5) | 39 (25.7) |
| Black | 39 (26.2) | 33 (21.7) |
| Hispanic | 53 (35.6) | 49 (32.2) |
| Asian | 19 (12.8) | 31 (20.4) |
| Low birthweight (%) | 4.7% | 9.9% |



## Fetal size differences per 1-week increase in Age Acceleration

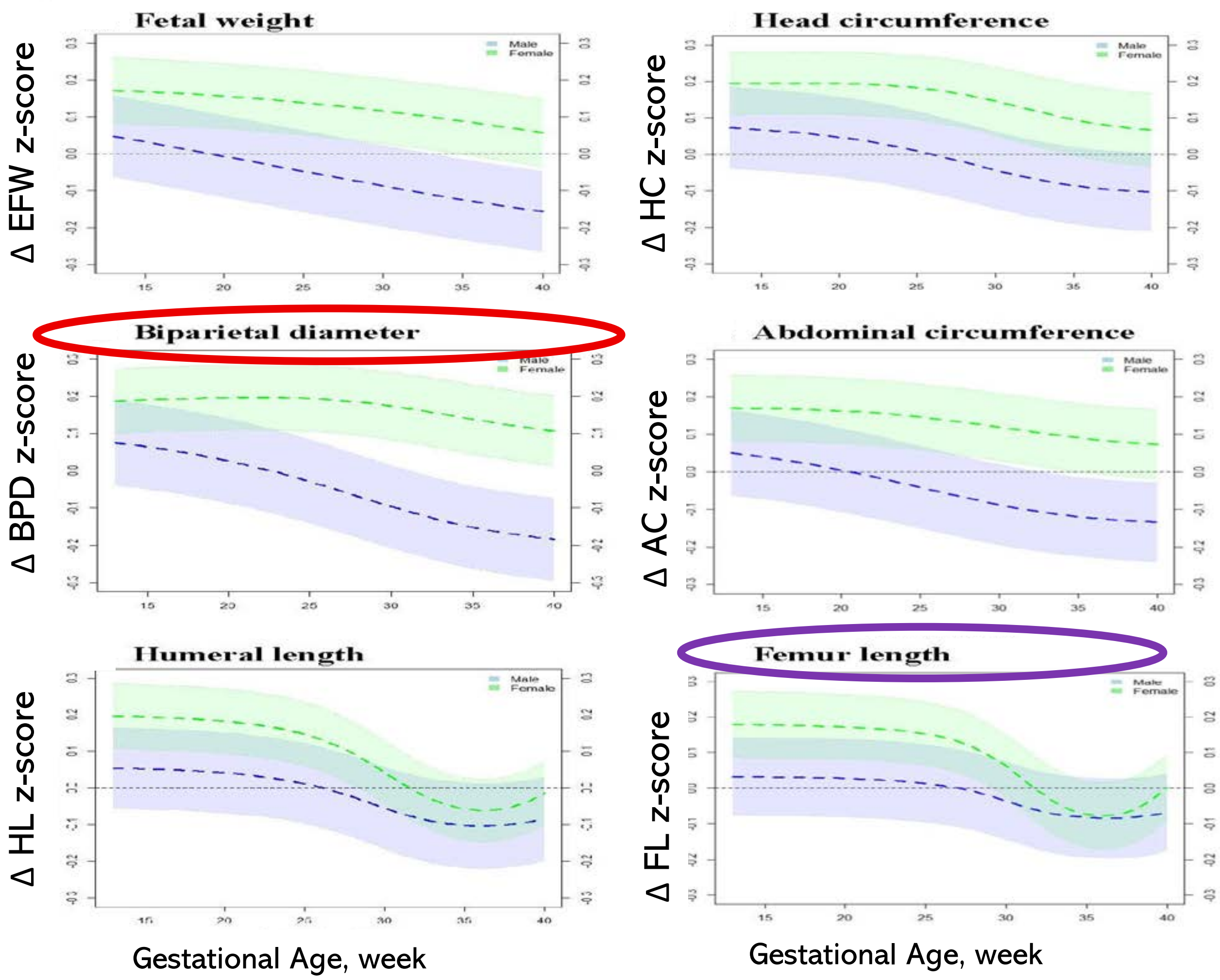
| | (95% CI) | *P* | (95% CI) | |
|---|---|---|---|---|
| Fetal weight, g | **-17.4 (-34.0, -0.8)** | **0.04** | **14.5 (0.9, 28.1)** | **0.04** |
| Head circumference, mm | -0.2 (-0.9, 0.6) | 0.68 | **1.2 (0.5, 1.8)** | **0.001** |
| Biparietal diameter, mm | -0.2 (-0.4, 0.1) | 0.21 | **0.4 (0.2, 0.6)** | **8.5e-5** |
| Abdominal circumference, mm | -0.8 (-1.9, 0.3) | 0.16 | **1.3 (0.4, 2.3)** | **0.01** |
| Humeral length, mm | -0.0 (-0.2, 0.2) | 0.85 | **0.2 (0.1, 0.4)** | **0.01** |
| Femur length, mm | 0.0 (-0.2, 0.2) | 0.97 | **0.2 (0.1, 0.3)** | **0.004** |

## Birth size difference per 1-week increase in Age Acceleration

| | Male neonate | | Female neonate | |
|---|---|---|---|---|
| | Estimate (95% CJ) | P | Estimate (95% CI) | |
| Birth weight, g | **-114.0 (-166.1, -61.9)** | **3.0e-5** | -31.9 (-70.2, 6.4) | 0.10 |
| Birth length, cm | **-0.4 (-0.7, -0.1)** | **0.004** | **-0.3 (-0.5, -0.1)** | **0.01** |
| Head circumference cm | **-0.3 (-0.5, -0.2)** | **2.7e-5** | -0.1 (-0.2, 0.0) | 0.10 |

Tekola-Ayele et al. *Aging* 2019

# Sex-specific associations differ based on gestational age, head bone *vs* long bone




Adjusted for maternal age, pre-pregnancy body mass index, race/ethnicity, marital status, educational status, health insurance ownership, parity, and mode of onset of labor.

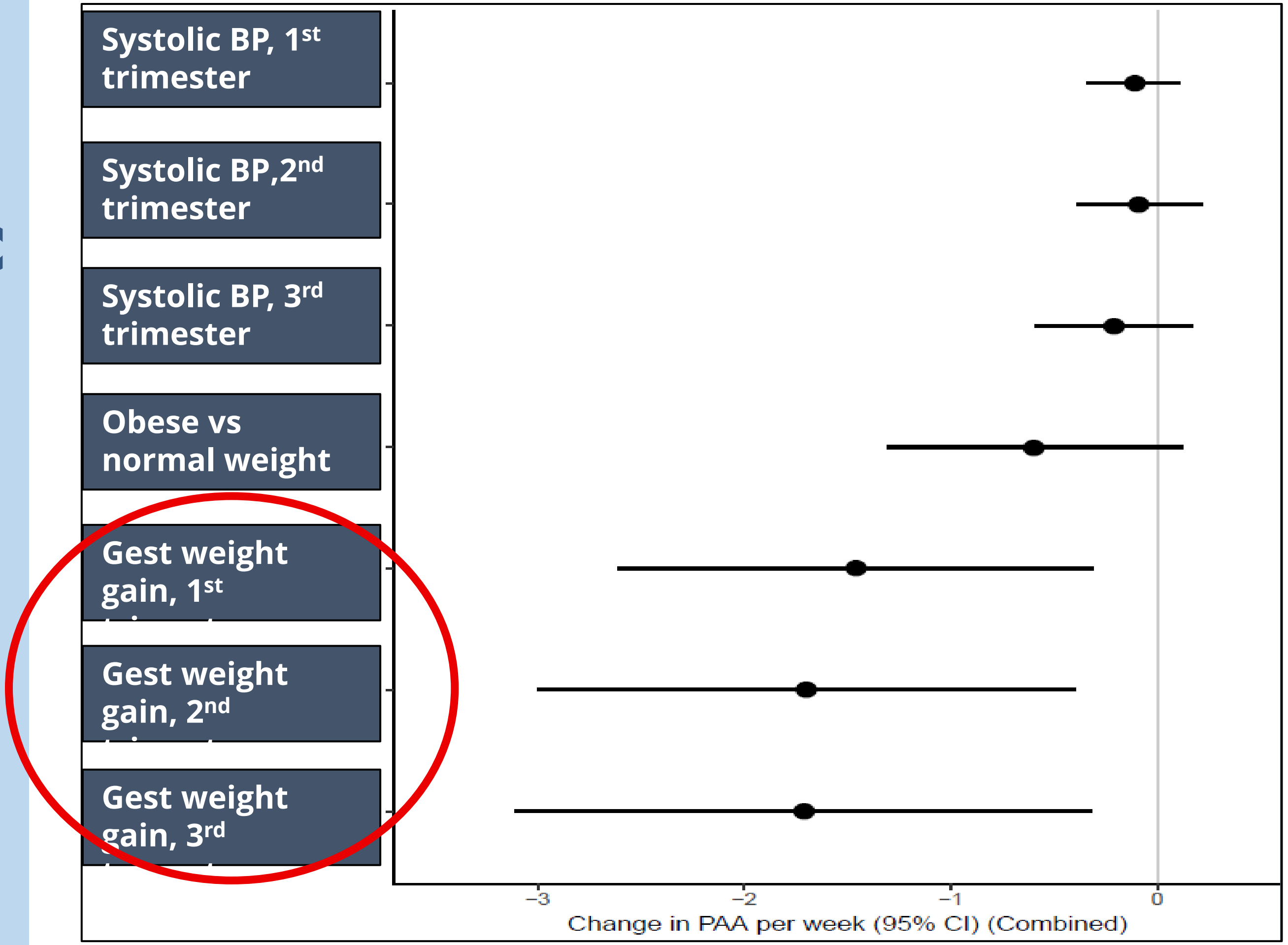
Males: inverse association with all growth measures

Females: positive association with head bones throughout gestation, with long bones until end of 2nd trimester

Tekola-Ayele et al. *Aging* 2019

## ❑ Maternal cardiometabolic factors & placental aging

- Blood pressure
- Pre-pregnancy obesity
- Dyslipidemia
- Gestational weight gain



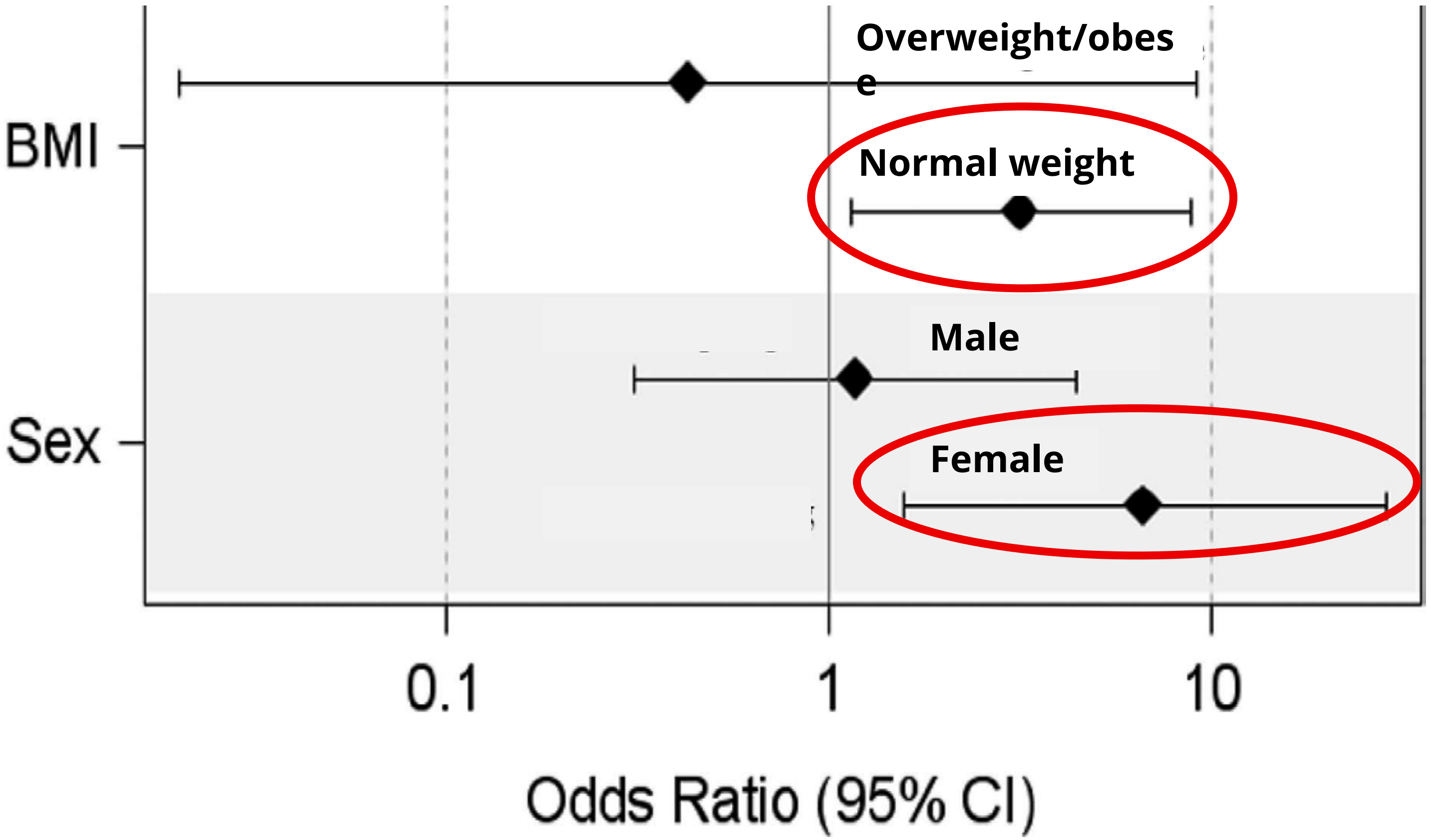

Adjusted for parity, health insurance, mode of onset of labor, marital status, educational status, preeclampsia status, and offspring sex

Workalemahu …Tekola-Ayele *J Dev orig Health Dis* 2020

# ❑ Maternal dyslipidemia & placental aging

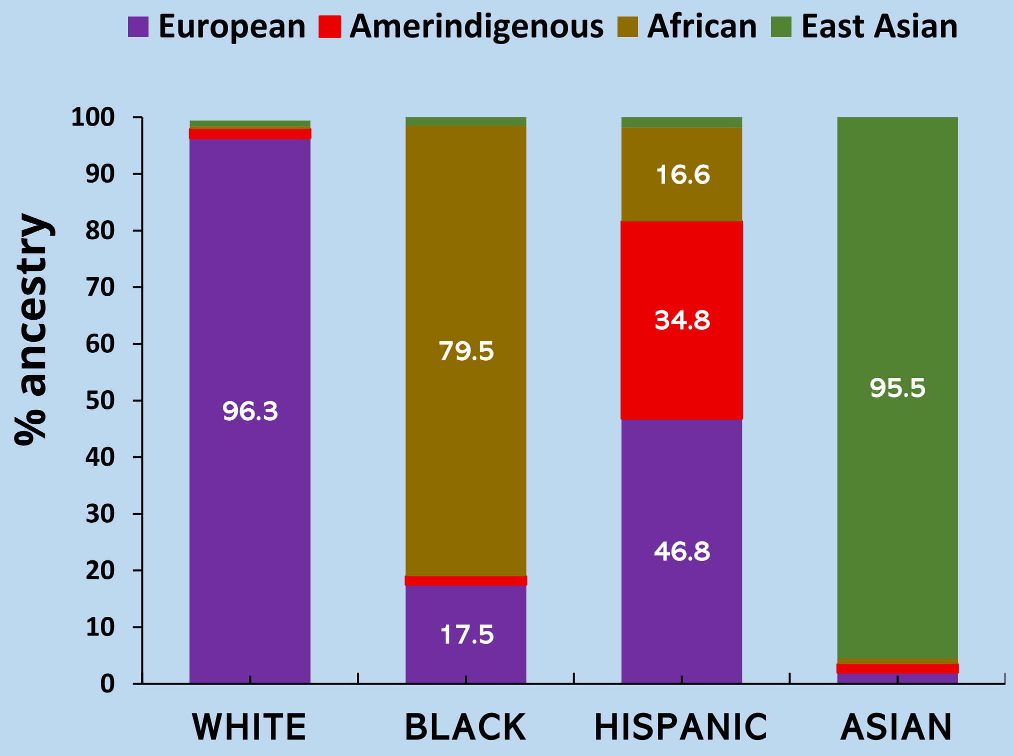
- HDL cholesterol
- LDL cholesterol
- Triglycerides
- Total cholesterol

**Positive placental age acceleration among women with low HDLc compared to normal HDLc**




Shrestha …Tekola-Ayele *Epigenetics* 2019

# ❑ Genetic ancestry & placental aging




| Women's genetic ancestry | ΔPAA, wk (95% CI) |
|---|---|
| **White** | |
| 10% higher European ancestry | 0.20 (-0.20, 0.60) |
| **Black** | |
| 10% higher African ancestry | -0.10 (-0.40, 0.20) |
| **Hispanic** | |
| 10% higher European ancestry | -0.10 (-0.30, 0.10) |
| 10% higher African ancestry | -0.20 (-0.50, 0.00) |
| 10% higher Native American ancestry | 0.20 (0.02, 0.40) |
| **Asian** | |
| 10% higher East Asian ancestry | -0.20 (-0.40, -0.04) |

| Offspring genetic ancestry | ΔPAA,wk (95% CI) |
|---|---|
| **White** | |
| 10% higher European ancestry | 0.10 (-0.20, 0.40) |
| **Black** | |
| 10% higher African ancestry | 0.05 (-0.20, 0.30) |
| **Hispanic** | |
| 10% higher European ancestry | -0.20 (-0.40, 0.10) |
| 10% higher African ancestry | -0.40 (-0.60, -0.20) * |
| 10% higher Native American ancestry | 0.30 (0.20, 0.50) * |
| **Asian** | |
| 10% higher East Asian ancestry | -0.10 (-0.30, 0.10) |

# Summary

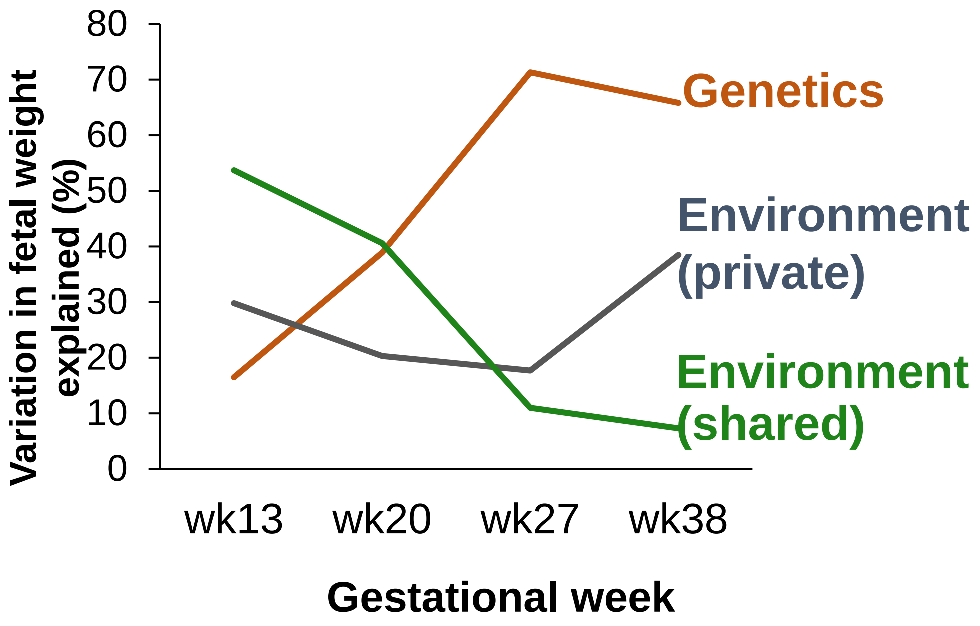
- Placental epigenetic aging may influence fetal growth trajectories, with distinct responses by sex

- Maternal dyslipidemia, higher gestational weight gain and genetic ancestry may drive placental aging

- Placental epigenetic clocks may be potential markers for in-utero exposures that influence pregnancy outcomes

# From GWAS … to regulatory function in placental aging

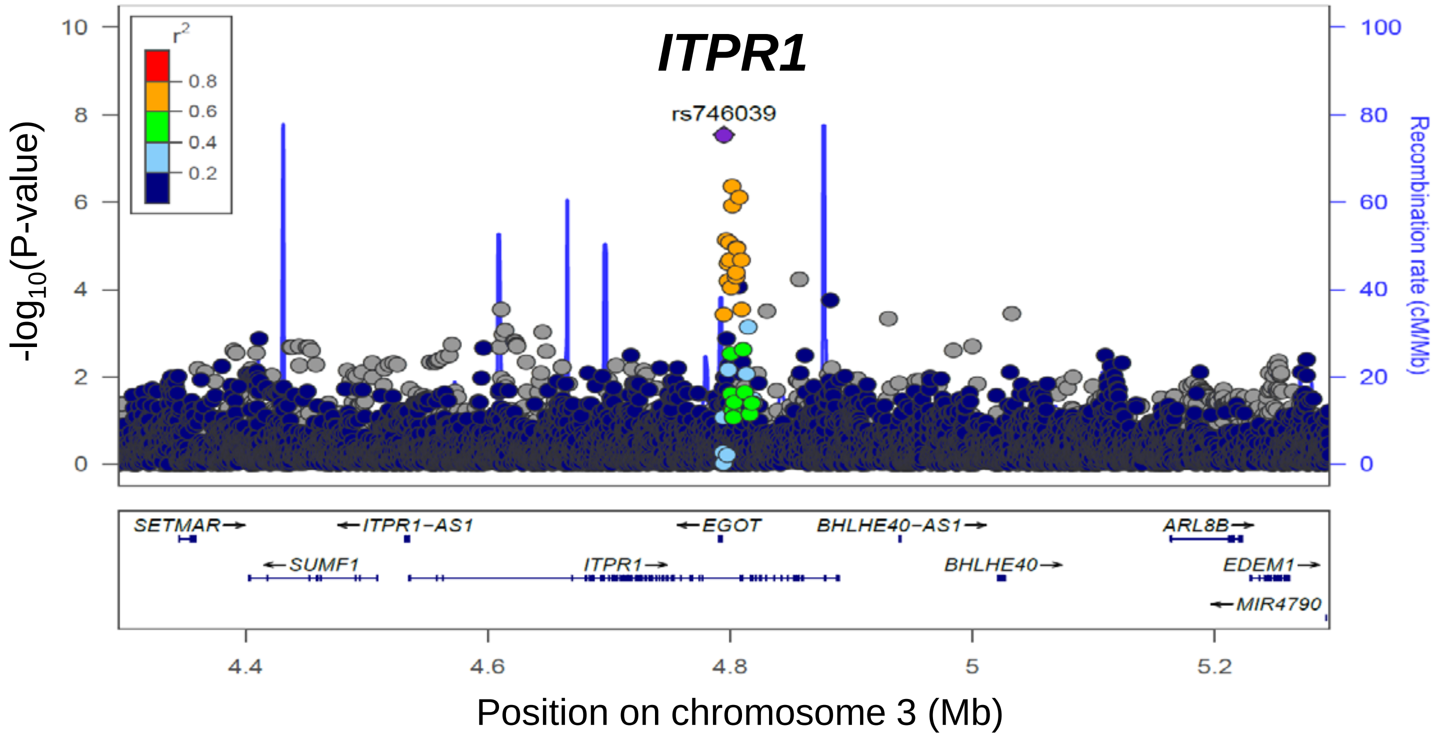
- **Genetic contributions on fetal growth vary by gestational age**




Workalemahu …Tekola-Ayele *Scient Reports* 2017

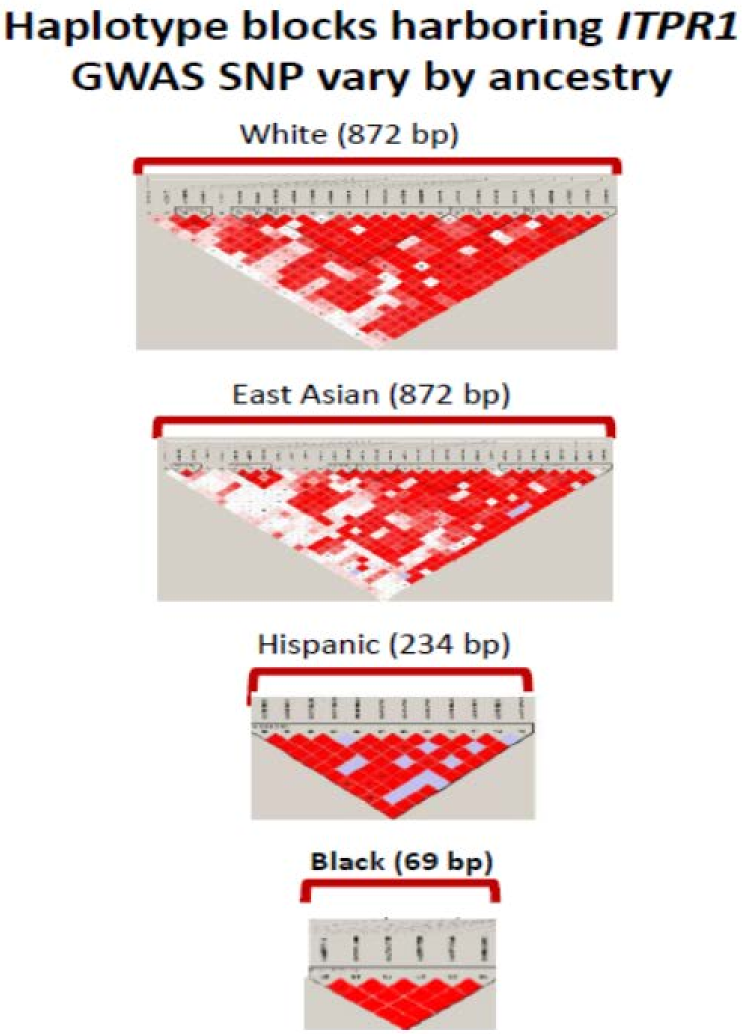
# *Trans*-ethnic GWAS (White, Black, Hispanic, Asian)

- ***ITPR1*** **locus associated with lower fetal weight at 27-32 wk**

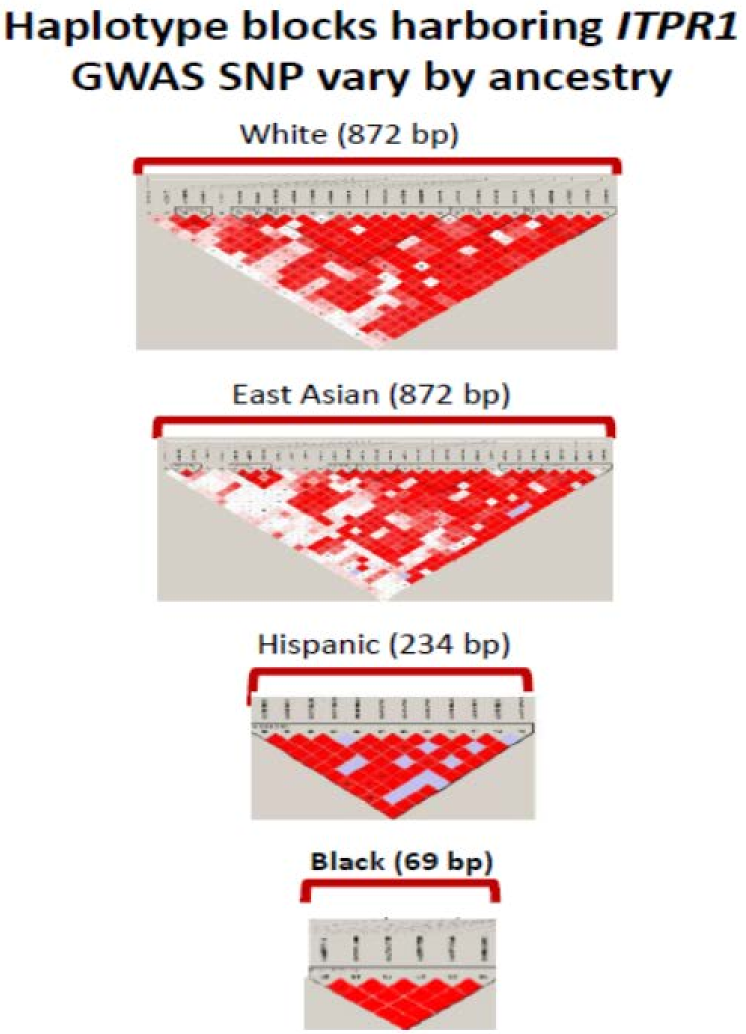



Tekola-Ayele et al. *PLOS Genetics* 2020

# From GWAS … to regulatory function in placental aging



# From GWAS … to regulatory function in placental aging



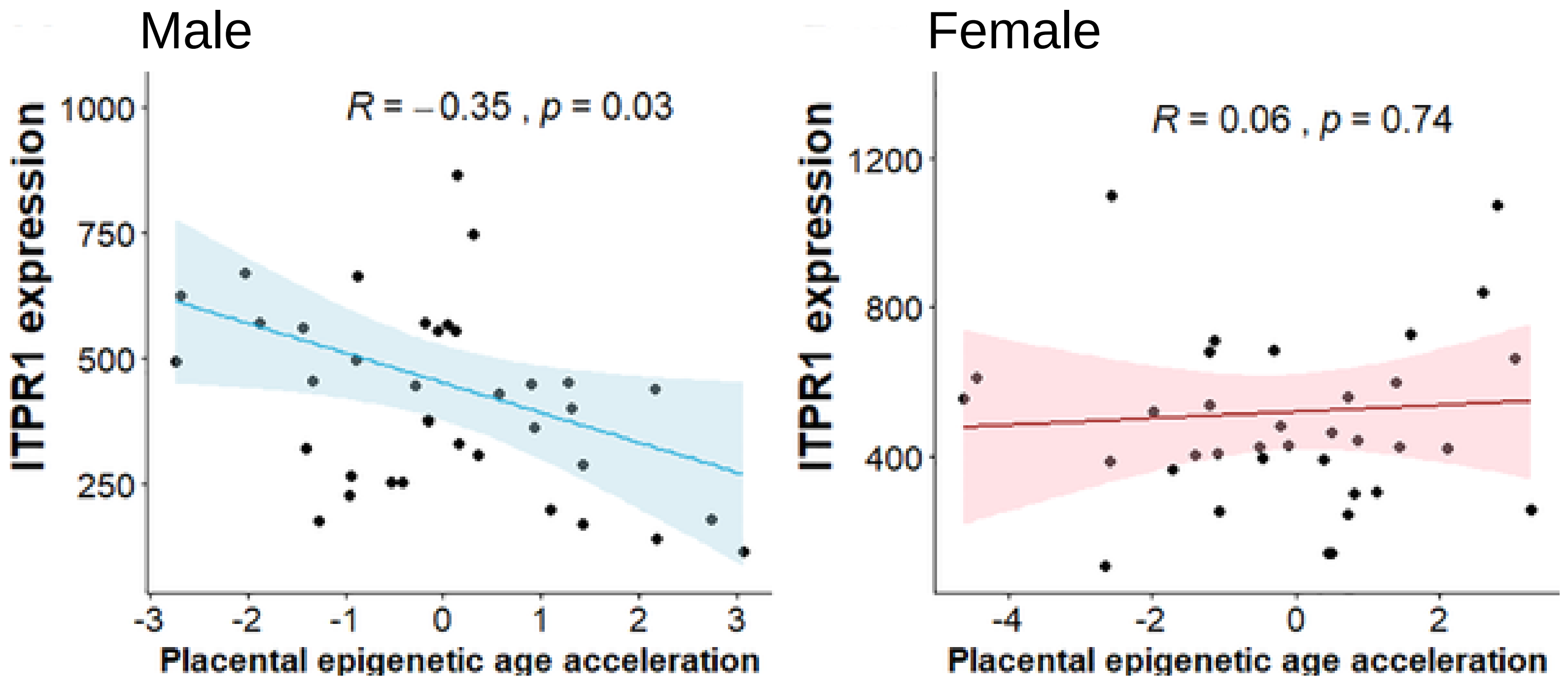

## ? Function

- induces calcium release from intracellular membranes
- mice *itpr1-/-* led to fetal growth retardation, decreased expression in placenta
- decreased expression in aged skeletal muscle

Fosket et al. *Physiol Rev* 2007, 87:593–58

# Hypothesis

**Decreased expression of *ITPR1* in placenta may lead to accelerated aging of the tissue, potentially linking the effect of the SNP on lower birthweight**



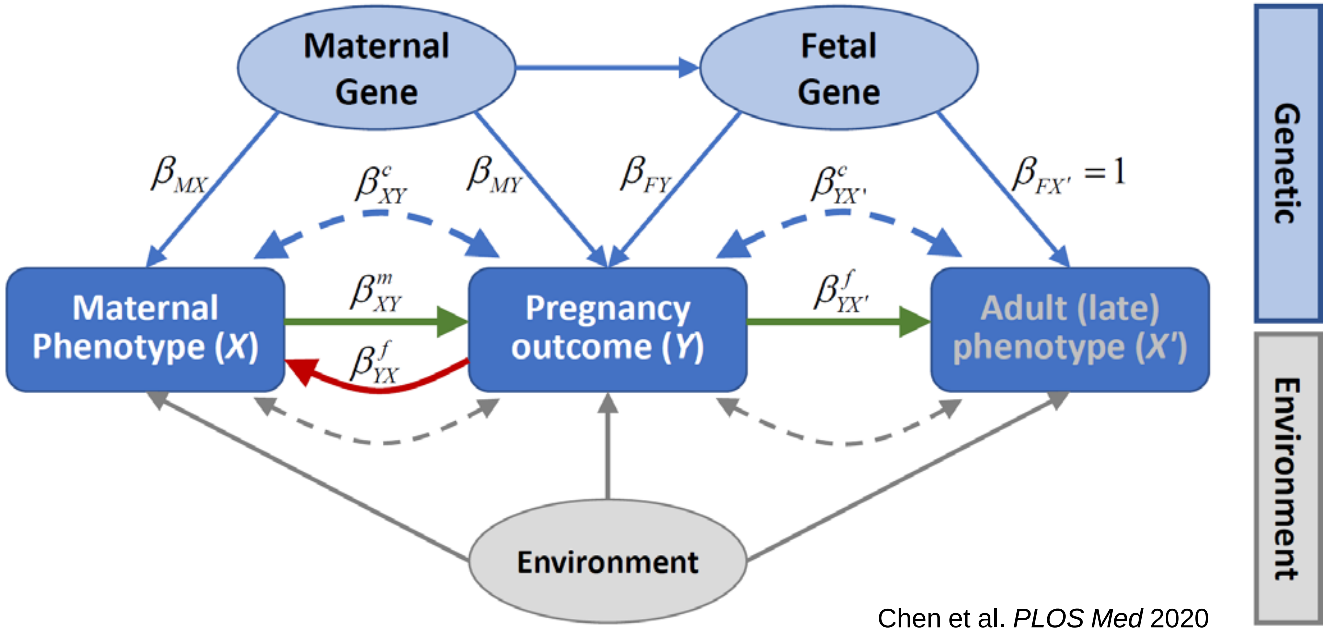

Tekola-Ayele et al. *PLOS Genetics* 2020

# Summary

- Genetic influences on fetal growth vary at different gestational weeks

- The *ITPR1* genetic locus may reduce fetal weight though a functional impact on placental aging – identifying the *in-utero* mechanism can inform molecular and clinical intervention targets

# Maternal cardiometabolic status → birth outcomes → future risk of CVD




Chen et al. *PLOS Med* 2020

- Maternal effect
- Fetal genetic effect
- Shared genes
- Fetal drive
- Environment

# Maternal cardiometabolic factors and birthweight in relation to placental methylome/transcriptome

Clinical Epigenetics

DNA methylation loci in placenta associated with birthweight and expression of genes relevant for early development and adult diseases

Tekola-Ayele et al. *Clinical Epigenetics* 2020

International Journal of Obesity

Genetics and Epigenetics

Placental DNA methylation changes associated with maternal prepregnancy BMI and gestational weight gain

Shrestha …Tekola-Ayele *IJO* 2019

Epigenomics

Early pregnancy dyslipidemia is associated with placental DNA methylation at loci relevant for cardiometabolic diseases

Ouidir …Tekola-Ayele *Epigenomics* 2020

Hypertension

Differential DNA Methylation in Placenta Associated With Maternal Blood Pressure During Pregnancy

Workalemahu …Tekola-Ayele *Hypertension* 2020

# Maternal cardiometabolic factors and birthweight in relation to placental methylome/transcriptome

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Ouidir ...Tekola-Ayele *Epigenomics* 2020

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*pertension* 2020

- **Relevant to biological processes involved in early development.**

- **several placental methylated and expressed genes are well-known cardiovascular disease loci in adults.**

# Maternal and fetal genetic variation and birthweight/CVD

- Maternal genetic variants:
- related to fetal growth (modulate *in-utero* environment)

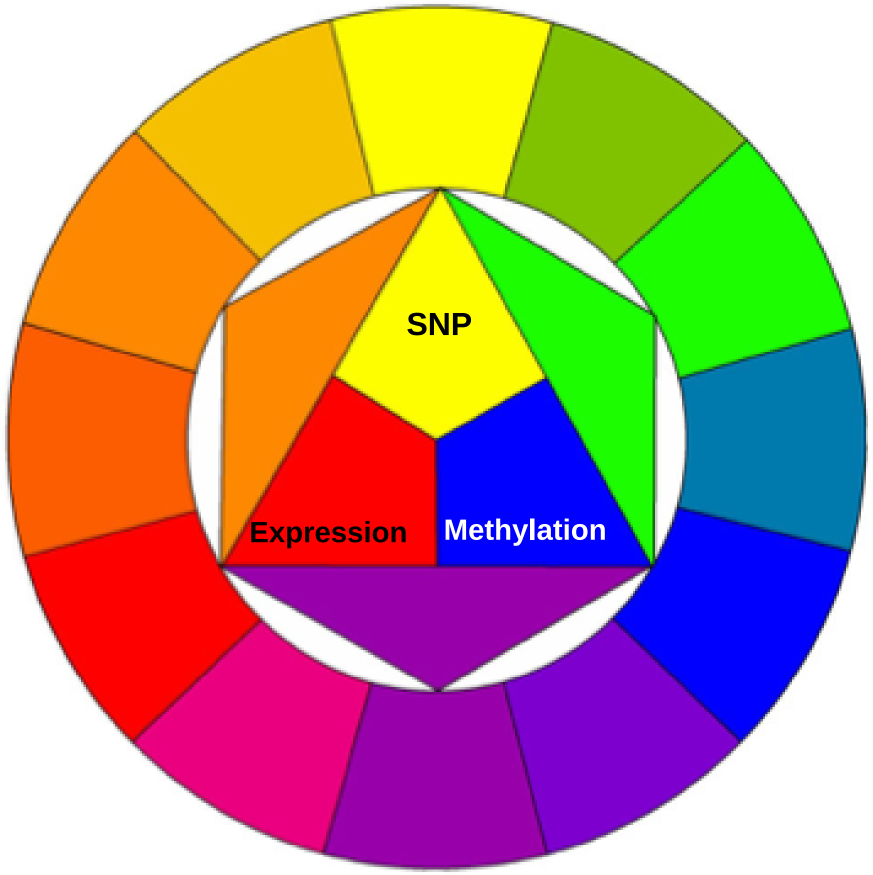
Polygenic risk for obesity, type 2 diabetes, lipids

Shrestha … Tekola-Ayele. *Obesity* 2019
Shrestha … Tekola-Ayele. *Front Genetics* 2018
Rahman … Tekola-Ayele. *JCEM* 2019
Ouidir … Tekola-Ayele. *J Clin Lipidology* 2019

- Fetal genetic variants: overlapping effect on birthweight & cardiometabolic diseases (pleiotropy)

Tekola-Ayele et al. *Hum Genomics* 2019
Tekola-Ayele et al. *Scient Reports* 2019

# Ongoing studies




## New Study

SPAN
Study of Pregnancy & Neonatal Health

**Aim 2 (PI: Tekola-Ayele). Genetics in fetal Growth and Placenta (gGAP)**

- Previous studies' focus: birth size, European ancestry populations, none on placenta
- Our focus: fetal size, placental aging, trans-ancestral (discovery in African Americans, n=4250 followed by trans-ethnic), multi-omics
- **Significance**: Insights into molecular mechanisms of early development, pregnancy complications & early origins of childhood & adult diseases

**Current fellows**
Marion Ouidir
Suvo Chatterjee

**Former fellows**
Tsegaselassie Workalemahu
Deepika Shrestha
Mohammad Rahman
Anthony Lee

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Ron Wapner
Jing Wu
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Several collaborators

Fetal Growth Study
NICHD

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NIMHD
NIH OD
NIDDK

EGG

SPAN