Genetic epidemiology of early growth-cardiometabolic disease links

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Epidemiology Branch, Division of Intramural Population Health Research
Prior to joining NICHD...

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

- **Socio-economic burden**
  - Tekola-Ayele et al. *BMC Med Eth* 2010

- **Develop clinical grading system**

- **Genetics**
  - Tekola-Ayele et al. *NEJM* 2012

- **Public health translation**
2011–2016 genetics of cardiometabolic diseases, population genetics

- **African Genome Variation Project**
  - *co-first author

- **Metabolic syndrome**

- **Type 2 diabetes**
  - Tekola-Ayele et al. *Pharmacogenomics J* 2014
Motivation

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

Barker et al. *Ped Perinat Epi* 1992

Bulik-Sullivan et al. *Nature Genetics* 2015
Research program

Goal: 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
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)
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

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.

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

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)

**Maternal DNA**
(n=2065)

**Methylation:**
- Infinium Human Methylation450 Beadchip
- Epigenetic age (62 CpGs)

**Genotyping:**
- HumanOmni2.5 Beadchip
- Fetal genetic ancestry

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

**Age Acceleration** = DNA methylation age – gestational age
### Characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Female offspring (n=149)</th>
<th>Male offspring (n=152)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal age, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30 yrs</td>
<td>89 (59.7)</td>
<td>93 (61.2)</td>
</tr>
<tr>
<td>30-35 yrs</td>
<td>44 (29.5)</td>
<td>45 (29.6)</td>
</tr>
<tr>
<td>≥35 yrs</td>
<td>16 (10.7)</td>
<td>14 (9.2)</td>
</tr>
<tr>
<td><strong>Gestational age at delivery, wk</strong></td>
<td>39.6 ± 1.1</td>
<td>39.4 ± 1.2</td>
</tr>
<tr>
<td><strong>Race/ethnicity, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>38 (25.5)</td>
<td>39 (25.7)</td>
</tr>
<tr>
<td>Black</td>
<td>39 (26.2)</td>
<td>33 (21.7)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>53 (35.6)</td>
<td>49 (32.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>19 (12.8)</td>
<td>31 (20.4)</td>
</tr>
<tr>
<td>Low birthweight (%)</td>
<td>4.7%</td>
<td>9.9%</td>
</tr>
</tbody>
</table>
## Fetal size differences per 1-week increase in Age Acceleration

<table>
<thead>
<tr>
<th></th>
<th>(95% CI)</th>
<th>P</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>-17.4 (-34.0, -0.8)</td>
<td>0.04</td>
<td>14.5 (0.9, 28.1)</td>
</tr>
<tr>
<td>Head circumference, mm</td>
<td>-0.2 (-0.9, 0.6)</td>
<td>0.68</td>
<td>1.2 (0.5, 1.8)</td>
</tr>
<tr>
<td>Biparietal diameter, mm</td>
<td>-0.2 (-0.4, 0.1)</td>
<td>0.21</td>
<td>0.4 (0.2, 0.6)</td>
</tr>
<tr>
<td>Abdominal circumference, mm</td>
<td>-0.8 (-1.9, 0.3)</td>
<td>0.16</td>
<td>1.3 (0.4, 2.3)</td>
</tr>
<tr>
<td>Humeral length, mm</td>
<td>-0.0 (-0.2, 0.2)</td>
<td>0.85</td>
<td>0.2 (0.1, 0.4)</td>
</tr>
<tr>
<td>Femur length, mm</td>
<td>0.0 (-0.2, 0.2)</td>
<td>0.97</td>
<td>0.2 (0.1, 0.3)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Male neonate</th>
<th>P</th>
<th>Female neonate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate (95% CI)</td>
<td></td>
<td>Estimate (95% CI)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>-114.0 (-166.1, -61.9)</td>
<td>3.0e-5</td>
<td>-31.9 (-70.2, 6.4)</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td>-0.4 (-0.7, -0.1)</td>
<td>0.004</td>
<td>-0.3 (-0.5, -0.1)</td>
</tr>
<tr>
<td>Head circumference cm</td>
<td>-0.3 (-0.5, -0.2)</td>
<td>2.7e-5</td>
<td>-0.1 (-0.2, 0.0)</td>
</tr>
</tbody>
</table>

Tekola-Ayele et al. *Aging* 2019
Sex-specific associations differ based on gestational age, head bone vs long bone

**Males**: inverse association with all growth measures

**Females**: positive association with head bones throughout gestation, with long bones until end of 2\textsuperscript{nd} trimester

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.

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

<table>
<thead>
<tr>
<th>Ancestry</th>
<th>ΔPAA, wk (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>White</td>
<td></td>
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<tr>
<td>10% higher European ancestry</td>
<td>0.20 (-0.20, 0.60)</td>
</tr>
<tr>
<td>10% higher African ancestry</td>
<td>-0.10 (-0.40, 0.20)</td>
</tr>
<tr>
<td>Hispanic</td>
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<tr>
<td>10% higher European ancestry</td>
<td>-0.10 (-0.30, 0.10)</td>
</tr>
<tr>
<td>10% higher African ancestry</td>
<td>-0.20 (-0.50, 0.00)</td>
</tr>
<tr>
<td>10% higher Native American ancestry</td>
<td>0.20 (0.02, 0.40)</td>
</tr>
<tr>
<td>Asian</td>
<td></td>
</tr>
<tr>
<td>10% higher East Asian ancestry</td>
<td>-0.20 (-0.40, -0.04)</td>
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#### Offspring genetic ancestry

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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

Variation in fetal weight explained (%)

Gestational week

wk13  wk20  wk27  wk38

Genetics

Environment (private)

Environment (shared)

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 \textit{ITPR1} genetic locus may reduce fetal weight though a functional impact on placental aging – identifying the \textit{in-utero} mechanism can inform molecular and clinical intervention targets
Maternal cardiometabolic status $\rightarrow$ birth outcomes $\rightarrow$ future risk of CVD

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

Chen et al. PLOS Med 2020
Maternal cardiometabolic factors and birthweight in relation to placental methylome/transcriptome

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

Placental DNA methylation changes associated with maternal prepregnancy BMI and gestational weight gain
Shrestha ...Tekola-Ayele *IJO* 2019

Early pregnancy dyslipidemia is associated with placental DNA methylation at loci relevant for cardiometabolic diseases
Ouidir ...Tekola-Ayele *Epigenomics* 2020

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

- 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

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

References:

- Shrestha ... Tekola-Ayele. *Obesity* 2019
- Shrestha ... Tekola-Ayele. *Front Genetics* 2018
- Rahman ... Tekola-Ayele. *JCEM* 2019
- Ouidir ... Tekola-Ayele. *J Clin Lipidology* 2019
- Tekola-Ayele et al. *Hum Genomics* 2019
- Tekola-Ayele et al. *Scient Reports* 2019
New Study

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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Una Grewal
Germaine Buck Louis
Stefanie Hinkle
Pauline Mendola
Jennifer Weck
Ron Wapner
Jing Wu
Xuehua Zeng
Several collaborators

**Funding**
NICHD, American Recovery and Reinvestment Act funding via contract numbers
HHSN275200800013C; HHSN275200800002I; HHSN27500006; HHSN27520080003IC;
HHSN275200800014C; HHSN275200800012C;
HHSN275200800028C; HHSN275201000009C
and HHSN27500008.
NIMHD
NIH OD
NIDDK