**Introduction:**
Age is a well-established risk factor for chronic diseases. However, the cellular and molecular changes associated with aging that are related to chronic disease initiation and progression are not well-understood. Recently, differential DNA methylation patterns that affect gene expression have been shown to be associated with aging. More specifically, age has been found to be associated with methylation status in pathways related to liver development and metabolism, inflammation, endothelial function, oxidation, and tumor suppression. Since epigenetic events provide a modifiable link between a gene’s expression and a resulting phenotype, unraveling the relationship between epigenetic mechanisms and cellular aging processes is crucial to understanding the origins of chronic diseases.

**Methods:**
Sample.
- African-Americans (age 39-94) in sibships from Phase II (Y2000-Y2004) of the Genetic Epidemiology Network of Arteriopathy (GENOA) study
- >2 siblings diagnosed with primary hypertension prior to age 60
- Evaluated for chronic disease risk factors via standardized interview, physical examination, and fasting blood sample

Measurement and quantification of DNA methylation.
DNA was isolated from stored peripheral blood leukocytes from 1,008 GENOA Phase II individuals, then bisulfite converted and hybridized to Illumina Infinium HumanMethylation27K BeadChips following standard protocol. The array was assessed for fluorescence intensities across methylated (M) and unmethylated (U) bead types, and two measures of methylation were calculated using the normalized signal intensities:

- Beta Value = \(\frac{\text{max}(M,0)}{\text{max}(U,0) + \text{max}(M,0) + 100}\)
- M-Value = \(\frac{\text{max}(M,0) + 1}{\text{max}(U,0) + 1}\)

- Sample size = 972 individuals after quality control for poor bisulfite conversion and outlying control probe values
- 26,428 autosomal CpG sites analyzed, after removal of 58 CpG sites significant with age based on M-Value and due to multimodality

**Statistical Analysis.**
- To examine the effects of age upon DNA methylation:
  \[E_x = \beta_a + \beta_1 \text{Age}_{x0} + W_x + \epsilon_x\]
- To evaluate how well epigenetic markers predicted age:
  \[\text{Age}_{x0} = \beta_0 + \beta_1 E_x + W_x + \epsilon_x\]

For participant i in sibship j on CpG site k, where E is the value of an epigenetic marker (Beta or M-Value), and W is the random effect for each sibship

Bonferroni-correction statistical significance for \(\alpha = 0.05\) at \(p < 1.89 \times 10^{-6}\)

- To assess how well the aggregation ofsignificant CpG sites predicted age, principal components (PCs) calculated across sites significant at Bonferroni correction for \(\alpha = 0.05\) in the second modeling set, above (Beta Values: 1,385 CpG sites; M-Values: 1,848 CpG sites)

- The top 5 PCs for the significant Beta and M-Values were modeled individually as predictors for age, and again in a multivariable mixed model:

- Age \(= \beta_0 + \beta_1 \text{PC1} + \beta_2 \text{PC2} + \beta_3 \text{PC3} + \beta_4 \text{PC4} + \beta_5 \text{PC5} + W_x + \epsilon_x\)

**Results:**

**Table 1. Baseline characteristics of GENOA Phase II Epigenetics participants**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (± SD)</th>
<th>Min</th>
<th>Max</th>
<th>N</th>
<th>Female</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66.26 (7.60)</td>
<td>39.26</td>
<td>94.74</td>
<td>972</td>
<td>687 (70.7%)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.15 (6.28)</td>
<td>16.41</td>
<td>60.35</td>
<td>968</td>
<td>Ever Smoker 405 (41.7%)</td>
<td></td>
</tr>
<tr>
<td>Systolic Blood, mm Hg</td>
<td>139.9 (21.42)</td>
<td>79</td>
<td>243</td>
<td>972</td>
<td>Coronary Heart Disease 69 (7.1%)</td>
<td></td>
</tr>
<tr>
<td>Diastolic Blood, mm Hg</td>
<td>78.33 (11.00)</td>
<td>45</td>
<td>121</td>
<td>972</td>
<td>Hypertension 902 (82.5%)</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>203.7 (42.08)</td>
<td>78.5</td>
<td>354</td>
<td>972</td>
<td>Diabetes 298 (30.8%)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>119.82 (64.20)</td>
<td>37</td>
<td>813.5</td>
<td>972</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>58.33 (17.99)</td>
<td>21</td>
<td>157.7</td>
<td>972</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Significant Findings:**

- **Introduction:**
  To assess how well the aggregation of significant CpG sites predicted age as outcome at \(p < 1.89 \times 10^{-6}\)

- The top 5 PCs explained 66% of the variance in epigenetic marker M-Values predicting age
- The top 5 epigenetic PCs explained 34% of variation in chronological age
- We also performed this analysis using Beta Values and results were similar

**Table 2. Association between top 10 principal components of 1,848 sites significant with age based on M-Value**

<table>
<thead>
<tr>
<th>PC</th>
<th>% Variation Explained</th>
<th>(\beta) (PC)</th>
<th>(R^2) (x100)</th>
<th>(\beta) (PC)</th>
<th>(R^2) (x100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46.11</td>
<td>-0.1469</td>
<td>18.55</td>
<td>-0.1756</td>
<td>13.59</td>
</tr>
<tr>
<td>2</td>
<td>10.69</td>
<td>0.1269</td>
<td>5.02</td>
<td>0.1545</td>
<td>9.21</td>
</tr>
<tr>
<td>3</td>
<td>5.16</td>
<td>-0.6942</td>
<td>25.36</td>
<td>-0.7524</td>
<td>25.36</td>
</tr>
<tr>
<td>4</td>
<td>2.39</td>
<td>-0.2906</td>
<td>15.17</td>
<td>-0.3456</td>
<td>15.17</td>
</tr>
<tr>
<td>5</td>
<td>1.96</td>
<td>0.1297</td>
<td>14.35</td>
<td>0.1271</td>
<td>13.93</td>
</tr>
<tr>
<td>6-10</td>
<td>4.86</td>
<td></td>
<td></td>
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</tbody>
</table>

**Conclusions:**
- The associations between age and methylation markers are so ubiquitous and strong across the age spectrum that we hypothesize that DNA methylation patterns are an important measure of cellular aging that underlies the association between chronic disease and chronological age.
- The increased density of negative t-values for unmethylated and hemi-methylated CpG sites indicates that unmethylated markers are increasingly less methylated with older age. The increased density of positive t-values for methylated CpG sites indicates that these methylated markers are increasingly more methylated with older age. (Figure 1)
- Changes in methylation may contribute to chronic diseases through a variety of mechanisms.
- Next steps:
  - Replication in other cohorts
  - Examining longitudinal trends
  - Examining other risk factor associations (e.g. inflammation)