DNA methylation of the ELOVL2, FHL2, KLF14, C1orf132, and TRIM59 genes in blood, saliva, and buccal swab samples

Sae Rom Hong, Sang-Eun Jung, Eun Hee Lee, Seung Min Lim, Kyoung-Jin Shin, Hwan Young Lee

Dept. of Forensic Medicine, Yonsei University College of Medicine, Korea

Nine Hallmarks of Aging

- Genomic instability
- Telomere attrition
- Epigenetic alterations
- Loss of proteostasis
- Deregulated nutrient sensing
- Mitochondrial dysfunction
- Cellular senescence
- Stem cell exhaustion
- Altered intercellular communication

López-Otín et al. Cell 2013
DNA Methylation Variation

Unsupervised clustering of average beta values in normal human tissues.

Age Prediction Based on DNA Methylation

Age = a + b × CpG1 + c × CpG2 + d × CpG3 + ...

Hannum et al. Mol Cell (2013)
### Multi-tissue Age Predictor

- Horvath’s model: 393 CpGs, less than 4 year error

![Horvath's model graphs]

### Tissue-specific Age Predictors

<table>
<thead>
<tr>
<th>Sample</th>
<th>Model</th>
<th>Genes</th>
<th>No. CpGs</th>
<th>Error (y)</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Weidner et al.</td>
<td>ITGA2B, ASPA, PDE4C</td>
<td>3</td>
<td>4.3</td>
<td>Pyro-sequencing</td>
</tr>
<tr>
<td></td>
<td>Zbiec-Piekarska et al.</td>
<td>ELOVL2</td>
<td>2</td>
<td>5.0</td>
<td>Pyro-sequencing</td>
</tr>
<tr>
<td></td>
<td>Zbiec-Piekarska et al.</td>
<td>ELOVL2, FHL2, KLF14, C1orf132, TRIM59</td>
<td>5</td>
<td>3.9</td>
<td>Pyro-sequencing</td>
</tr>
<tr>
<td></td>
<td>Park et al.</td>
<td>ELOVL2, ZNF423, CCDC102B</td>
<td>3</td>
<td>3.4</td>
<td>Pyro-sequencing</td>
</tr>
<tr>
<td>Saliva</td>
<td>Bocklandt et al.</td>
<td>EDARADD, TOM1, NPTX2</td>
<td>3</td>
<td>5.2</td>
<td>27k array</td>
</tr>
<tr>
<td></td>
<td>Hong et al.</td>
<td>SST, CNGA3, KLF14, TSSK6, TBR1, SLC12A5, PTPN7</td>
<td>7</td>
<td>3.1</td>
<td>SNaPshot</td>
</tr>
<tr>
<td>Semen</td>
<td>Lee et al.</td>
<td>TTCyB, NOX4, unknown</td>
<td>3</td>
<td>5.4</td>
<td>SNaPshot</td>
</tr>
<tr>
<td>Blood, teeth</td>
<td>Bekaeer et al.</td>
<td>ASPA, PED4C, ELOVL2, EDARADD</td>
<td>4</td>
<td>4.9</td>
<td>Pyro-sequencing</td>
</tr>
<tr>
<td>Teeth</td>
<td>Giuliani et al.</td>
<td>ELOVL2, FHL2, PENK</td>
<td>5.13</td>
<td>1.2-7.1</td>
<td>EpiTyper</td>
</tr>
</tbody>
</table>
Independent Validation of Blood Models

- Zbieć-Piekarska’s model: ELOVL2, FHL2, KLF14, C1orf132, TRIM59
- Analysis platform: Pyrosequencing

300 Polish

Spearman’s rho = 0.972
MAD = 3.90

100 Koreans

Spearman’s rho = 0.940
MAD = 4.18


Independent Validation of Blood Models

- Another model with 5 CpGs explaining the highest% of age variance in each gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Target (GRCh37)</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td></td>
<td>-10.403</td>
</tr>
<tr>
<td>ELOVL2</td>
<td>6:12044861</td>
<td>0.612</td>
</tr>
<tr>
<td>FHL2</td>
<td>2:1050515739</td>
<td>0.465</td>
</tr>
<tr>
<td>KLF14</td>
<td>7:130419316</td>
<td>0.330</td>
</tr>
<tr>
<td>C1orf132</td>
<td>1:207997026</td>
<td>-0.168</td>
</tr>
<tr>
<td>TRIM59</td>
<td>3:160617977</td>
<td>0.428</td>
</tr>
</tbody>
</table>

Change in Analysis Platform

- Multiplex methylation SNAPSHOT for age prediction in blood

Yellow: methylation
Red: non-methylation
Blue: methylation
Green: non-methylation

Blue: methylation
Green: non-methylation

ELOVL2
FHL2
KLF14
C1orf132
TRIM59

20s
30s
40s
50s
60s

FORWARD

\[ \% \text{methyl} = \frac{\text{C intensity}}{\text{(C+T) intensity}} \times 100 \]

REVERSE

\[ \% \text{methyl} = \frac{\text{G intensity}}{\text{(G+A) intensity}} \times 100 \]
Change in Analysis Platform

Pyrosequencing

Methylation SNaPshot

\[ \text{MAD} = 3.38 \]

\[ \text{MAD} = 4.37 \]

\[ \rho = 0.967 \]

Methylation of Blood, Saliva and Buccal Swab

**ELOVL2**

\[ R^2 = 0.7789 \text{ (blood)} \]

\[ R^2 = 0.6921 \text{ (saliva)} \]

\[ R^2 = 0.6921 \text{ (blood)} \]

\[ R^2 = 0.5031 \text{ (saliva)} \]

\[ R^2 = 0.4995 \text{ (buccal swab)} \]

**FHL2**

\[ R^2 = 0.5901 \text{ (blood)} \]

\[ R^2 = 0.5031 \text{ (saliva)} \]

\[ R^2 = 0.5197 \text{ (buccal swab)} \]

**KLF14**

\[ R^2 = 0.674 \text{ (blood)} \]

\[ R^2 = 0.913 \text{ (saliva)} \]

\[ R^2 = 0.4995 \text{ (buccal swab)} \]

**C1orf132**

\[ R^2 = 0.7814 \text{ (blood)} \]

\[ R^2 = 0.4703 \text{ (saliva)} \]

\[ R^2 = 0.3161 \text{ (buccal swab)} \]

**TRIM59**

\[ R^2 = 0.5786 \text{ (blood)} \]

\[ R^2 = 0.4389 \text{ (saliva)} \]

\[ R^2 = 0.2074 \text{ (buccal swab)} \]
Summary

- DNA methylation at 5 CpG sites from the ELOVL2, FHL2, KLF14, C1orf132, and TRIM59 genes was investigated in samples from blood, saliva, and buccal swabs using a multiplex methylation SNaPshot assay.
- An age prediction model trained on 240 samples including 80 of each blood, saliva and buccal swab samples exhibited high correlation between predicted and chronological ages with a MAD of 3.38 years.
- The model showed a MAD of 3.54 years in a validation set of 60 samples including 20 of each blood, saliva and buccal swab samples.
- These results suggest that these age-associated markers are less tissue-specific than others.
Acknowledgment

Kyoung-Jin Shin  Sang-Eun Jung  Eun Hee Lee  Sae Rom Hong

Bomin Kim  Mi Hyeon Moon  SeungMin Lim  Yelim Kwon