DNA methylation profiles in peripheral blood CD4$^+$ lymphocytes versus brain

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Disclosures

- I have no disclosure.
Motivation

DNA methylation
DNA methylation and AD
Complexity of interrogating DNA methylation
Objectives

Methods

Subjects
DNA methylation measures
Statistical analysis

Results

Global features
Correlation and difference
Comparison of associations in target CpG sites

Conclusions

Acknowledgment
DNA methylation

- DNA methylation is a most common epigenetic marker;
- Addition of a methyl (CH3) group to DNA nucleotides.

(Qiu. 2006 Nature)
DNA methylation

- DNA methylation is essential in regulating gene expression, cell differentiation and development;
- Typically it acts to suppress gene transcription
DNA methylation

- DNA methylation is essential in regulating gene expression, cell differentiation and development;
- Typically it acts to silence/suppress gene transcription

Agouti (ASPI) mice (Waterland and Jirtle. 2003 *Mol Cell Biol*)
DNA methylation and AD

- Aberrant methylation alterations are a/w AD.
  - Neuronal immunoreactivity for DNA methylation markers are reduced in AD (Mastroeni et al. 2010 Neurobiol Aging).
  - Early alterations in DNA methylation in 11 loci are associated with AD pathology (De Jager et al. 2014 Nat Neurosci).
  - DNA methylation in AD loci are implicated in pathologic AD diagnosis (Yu et al. 2015 JAMA Neurol).
Complexity of interrogating DNA methylation

Tissue/Cell type specificity

► The utility of interrogating peripheral tissue for a role of DNA methylation in neurodegeneration is unclear;

DNA methylation pattern change over time

► Global methylation decreases with age (Heyn et al. 2012 Proc Natl Acad Sci U S A);
► Fastest changes occur during the prenatal period, slow down markedly after birth and slow further with aging (Numata et al. 2012 Am J Hum Genet);
► Global methylation changes over time, but some increase and some decrease (Bjornsson et al. 2008 JAMA).
Objectives

- Compare global features of DNA methylation;
- Examine correlations and paired difference at individual CpG sites;
- Test associations of brain methylation with AD pathology using blood data.
Methods

Subject characteristics (N=41)

- ROS & MAP subjects with methylation measured three times (T1, T2, and death);
- Age at death: 89.6 years (SD=4.9);
- 27 (65.9%) were females;
- Education: 15.2 years (SD=3.5);
- Years between T1 and T2 (Mean=7.5, SD=4.1);
- Years between T2 and death (Mean=0.9, SD=0.7);
- 25 w/dementia at death;
- 30 w/ pathologic AD diagnosis.
Methods

DNA methylation measures

- Blood data are from CD4$^+$ lymphocytes; brain data from DLPFC;
- Infinium HumanMethylation450 BeadChip;
- 420,132 CpG sites in autosomes;
Statistical analyses

- Global features are captured by average methylation level;
- Pearson correlations and paired $t$-tests;
- Linear regression test the association with AD pathology (neuritic plaque).
Bimodal distribution of average DNA methylation across genome

**Global features**
- Correlation and difference
- Comparison of associations in target CpG sites

**Motivation**
**Methods**
**Results**
**Conclusions**
**Acknowledgment**
Distribution by island features

[Graph showing distribution by island features]
Distribution by gene features
DNA methylation at random CpG sites
Pair plot of DNA methylation at random CpG sites
Pair plot of DNA methylation at random CpG sites
Difference between T1 and T2
Difference between T2 and brain
### Top CpGs associated with AD

<table>
<thead>
<tr>
<th>CpG</th>
<th>CHR</th>
<th>Est</th>
<th>( p )</th>
<th>Nearby genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg11724984</td>
<td>12</td>
<td>3.02</td>
<td>( 4.76 \times 10^{-9} )</td>
<td>RNF34, KDM2B</td>
</tr>
<tr>
<td>cg23968456</td>
<td>10</td>
<td>4.97</td>
<td>( 3.97 \times 10^{-10} )</td>
<td>CDH23, C10orf105, C10orf54</td>
</tr>
<tr>
<td>cg15821544</td>
<td>1</td>
<td>3.52</td>
<td>( 1.17 \times 10^{-7} )</td>
<td>SLC2A1, FLJ32224</td>
</tr>
<tr>
<td>cg16733298</td>
<td>16</td>
<td>2.75</td>
<td>( 5.24 \times 10^{-8} )</td>
<td>COQ7, ITPRIPL2</td>
</tr>
<tr>
<td>cg22962123</td>
<td>7</td>
<td>1.7</td>
<td>( 1.12 \times 10^{-7} )</td>
<td>HOXA1, HOTAIRM1, HOXA2, AK291164, HOXA3, ...</td>
</tr>
<tr>
<td>cg13076843</td>
<td>17</td>
<td>2.35</td>
<td>( 1.68 \times 10^{-9} )</td>
<td>UBE2O, AANAT, RHBDF2, AX747521, CYGB, PRCD</td>
</tr>
<tr>
<td>cg25594100</td>
<td>7</td>
<td>3.15</td>
<td>( 2.54 \times 10^{-11} )</td>
<td>FOXP1, AP5Z1 (KIAA0415), RADIL</td>
</tr>
<tr>
<td>cg00621289</td>
<td>21</td>
<td>3.5</td>
<td>( 6.48 \times 10^{-8} )</td>
<td>PCNT, DIP2A</td>
</tr>
<tr>
<td>cg19803550</td>
<td>17</td>
<td>4.36</td>
<td>( 1.04 \times 10^{-8} )</td>
<td>PRPF8, TLCD2, MIR22HG, AF070569, MIR22, WDR81, ...</td>
</tr>
<tr>
<td>cg03169557</td>
<td>16</td>
<td>4.86</td>
<td>( 3.99 \times 10^{-10} )</td>
<td>ANKR11, SPG7, SNORD68, RPL13, CPNE7</td>
</tr>
<tr>
<td>cg05066959</td>
<td>8</td>
<td>2.69</td>
<td>( 7.13 \times 10^{-14} )</td>
<td>AGPAT6, NKX6-3, JA429246, ANK1</td>
</tr>
<tr>
<td>cg05810363</td>
<td>17</td>
<td>2.95</td>
<td>( 3.68 \times 10^{-10} )</td>
<td>UBE2O, AANAT, RHBDF2, AX747521, CYGB, PRCD</td>
</tr>
<tr>
<td>cg22883290</td>
<td>2</td>
<td>4.41</td>
<td>( 3.73 \times 10^{-8} )</td>
<td>BIN1</td>
</tr>
<tr>
<td>cg02308560</td>
<td>19</td>
<td>2.19</td>
<td>( 3.06 \times 10^{-8} )</td>
<td>CNN2, ABCA7, HMHA1, POLR2E, GPX4, SBNO2</td>
</tr>
</tbody>
</table>

(De Jager et al. 2014 *Nat Neurosci* Table 1)
Comparison of associations with neuritic plaques between blood and brain

<table>
<thead>
<tr>
<th>CpG</th>
<th>Est (brain)</th>
<th>p (brain)</th>
<th>Est (T1)</th>
<th>p (T1)</th>
<th>Est (T2)</th>
<th>p (T2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg11724984</td>
<td>4.48421</td>
<td>0.008</td>
<td>-4.47558</td>
<td>0.761</td>
<td>-37.32927</td>
<td>0.027</td>
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<tr>
<td>cg23968456</td>
<td>8.92299</td>
<td>0.014</td>
<td>0.29185</td>
<td>0.985</td>
<td>3.86053</td>
<td>0.826</td>
</tr>
<tr>
<td>cg15821544</td>
<td>6.05472</td>
<td>0.025</td>
<td>-18.28297</td>
<td>0.023</td>
<td>0.62902</td>
<td>0.931</td>
</tr>
<tr>
<td>cg16733298</td>
<td>4.53274</td>
<td>0.006</td>
<td>5.07275</td>
<td>0.378</td>
<td>1.84116</td>
<td>0.708</td>
</tr>
<tr>
<td>cg22962123</td>
<td>2.43252</td>
<td>0.036</td>
<td>12.06577</td>
<td>0.144</td>
<td>-6.71269</td>
<td>0.165</td>
</tr>
<tr>
<td>cg13076843</td>
<td>2.66407</td>
<td>0.033</td>
<td>-3.62692</td>
<td>0.599</td>
<td>-7.97847</td>
<td>0.233</td>
</tr>
<tr>
<td>cg25594100</td>
<td>5.98322</td>
<td>0.001</td>
<td>-14.31339</td>
<td>0.111</td>
<td>-2.78358</td>
<td>0.778</td>
</tr>
<tr>
<td>cg00621289</td>
<td>7.65410</td>
<td>0.003</td>
<td>2.54079</td>
<td>0.504</td>
<td>-9.64311</td>
<td>0.048</td>
</tr>
<tr>
<td>cg19803550</td>
<td>7.79731</td>
<td>0.002</td>
<td>3.19687</td>
<td>0.727</td>
<td>2.19308</td>
<td>0.865</td>
</tr>
<tr>
<td>cg03169557</td>
<td>7.01246</td>
<td>0.006</td>
<td>-5.84686</td>
<td>0.369</td>
<td>-25.30343</td>
<td>0.180</td>
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<tr>
<td>cg05066959</td>
<td>2.35733</td>
<td>0.091</td>
<td>-4.72639</td>
<td>0.062</td>
<td>0.10942</td>
<td>0.969</td>
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<tr>
<td>cg05810363</td>
<td>3.50167</td>
<td>0.052</td>
<td>-11.12024</td>
<td>0.487</td>
<td>-13.72232</td>
<td>0.576</td>
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<tr>
<td>cg22883290</td>
<td>8.23246</td>
<td>0.006</td>
<td>-11.02340</td>
<td>0.387</td>
<td>4.79474</td>
<td>0.678</td>
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<tr>
<td>cg02308560</td>
<td>0.89543</td>
<td>0.493</td>
<td>1.24928</td>
<td>0.606</td>
<td>2.38878</td>
<td>0.380</td>
</tr>
</tbody>
</table>
Conclusions

- Global features of DNA methylation are largely conserved between blood and brain;
- Concordance between bloods, and more diffuse between blood and brain;
- Approximately half of the CpG sites differ between blood and brain; a majority show lower methylation level in brain.
- Brain methylation association with AD pathology are not replicated in blood.
Acknowledgment

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