Update on DNA methylation alterations at birth from pregnancy folate intake and smoking from the California Childhood Leukemia Study

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Outline

• Relationship of folic acid and childhood leukemogenesis

• Folate intake in pregnancy and DNA methylation at birth
How might folate influence DNA methylation and leukemia?

Methionine Synthesis & SAM-Mediated Methyllations

Folic Acid

DNA Synthesis

DNA Methylation

Methionine
→ SAM
→ 5-Methyl THF
→ Homocysteine

DHF

THF

5,10-Methylene THF

MS

DHFR

THFR

5-Methyl THF

Folate

THM(D)
Subtypes of childhood acute lymphoblastic leukemia

INFANTS
< 1 YR

CHILDREN
1 – 15 YRS

ADULTS
16+ YRS

- Hyperdiploidy
- t(12;12) TEL-AML1
- 11q23 MLL
- Others
- t(9;22) BCR-ABL1
Leukemia exhibits profound DNA methylation changes

California Childhood Leukemia Study (CCLS)

- Case-control study (1995-2014)
- Ultra-rapid ascertainment of over 1,400 incident cases from 16 hospitals throughout California
- 1,426 controls selected from the California birth registry
- 47% Hispanic children
Maternal Folate and Vitamin Intake

- A modified version of the Block Food Frequency Questionnaire (FFQ) was administered during the in-home interviews to assess the pre/peri-pregnancy diet of the mother.

- Daily intake in micrograms per day of dietary folate/vitamin equivalents (mcg DFEs) was calculated from supplement use and dietary intake.
### Total Nutrient Intake from Food and Supplements & Childhood ALL

N= 645 cases, 854 controls

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Principal Component</strong></td>
<td>0.91 (0.84-0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Folate</strong> (100 DFE/day)</td>
<td>0.97 (0.94-1.01)</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Vitamin B12</strong> (1 µg/day)</td>
<td>0.96 (0.93-0.99)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Vitamin B6</strong> (1 mg/day)</td>
<td>0.89 (0.79-0.99)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Riboflavin</strong> (1 mg/day)</td>
<td>0.88 (0.77-0.99)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Methionine</strong> (1 g/day)</td>
<td>0.89 (0.73-1.10)</td>
<td>0.28</td>
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</tbody>
</table>

Conditional logistic models adjusted for mother’s ethnicity, father’s education, mother’s education, household income, maternal age at child’s birth, and energy intake.

*a The principal component represents the combined dietary intake of folate, vitamins B12 and B6, riboflavin and methionine from food and supplements.
Maternal Supplementation with Folic Acid and Other Vitamins and Risk of Leukemia in Offspring
A Childhood Leukemia International Consortium Study

Catherine Metayer, Elizabeth Milne, John D. Dockerty, Jacqueline Clavel, Maria S. Pombo-de-Oliveira, Catharina Wesseling, Logan G. Spector, Joachim Schüz, Eleni Petridou, Sameena Ezzat, Bruce K. Armstrong, Jérémie Rudant, Sergin Knifman, Peter Kaatsch, Marla Moschows, Wafaa M. Rashed, Steve Selvin, Kathryn McCauley, RayJean J. Hung, Alice Y. Kang, and Claire Ingrassia-Rivard

Epidemiology 2014;25: 811–822
Childhood Leukemia International Consortium
Pooled Analyses
Childhood ALL

<table>
<thead>
<tr>
<th>Parental education</th>
<th>Overallb</th>
<th>Vitamins (Any Time)</th>
<th>Folic Acid (Any Time)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Studies</td>
<td>No. Exposed</td>
<td>Test for Interaction</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>Cases</td>
<td>OR (95% CI)a</td>
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<tr>
<td>Overallb</td>
<td>12</td>
<td>6640</td>
<td>4336</td>
</tr>
<tr>
<td>None/Primary</td>
<td>12</td>
<td>873</td>
<td>447</td>
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<tr>
<td>Secondary</td>
<td>12</td>
<td>2649</td>
<td>1879</td>
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<tr>
<td>Tertiary</td>
<td>12</td>
<td>3118</td>
<td>2010</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, ethnicity, parental education, and study. OR for parental education is adjusted for age, sex, ethnicity, and study.

Metayer, et al  Epidemiology 2014
Neonatal Blood Spots: Guthrie Cards

Sample of blood taken immediately after birth (1-3 days)

Available for children born in California (3+ decades)

Used to trace back leukemia origin to fetal period
Folate and DNA methylation at birth: methods

• IlluminaInfinum HM450K array:
  – Polymorphic and SNP-related probes excluded
  – Functional Normalization (Fortin et al.)
  – Cell mixture estimation (WBC Inf., Houseman et al.)
• Folate exposure assessed by FFQ of the mothers about the peri-conception period
  – Total folate = food + supplementation
• Statistical analysis: locus-by-locus analysis
  – Logit transformation of beta-values
  – \text{lm}(\text{M-value} \sim \text{folate} + \text{sex} + \text{gestational age} + \text{cell-mixture estimates} + \text{race})
  – data were resampled 1,000 times with replacement (i.e., bootstrapping) in each set, with the locus-by-locus model run on each bootstrap sample
• First discovery set (n=176)
• Replication in independent sample from the same population (n=167)
Results

- **Clinical characteristics in the discovery set:**
  - Folate: range from 54.2 to 2229.0, mean=678.2 and sd=430.4 [mg/d]
  - 57.8% of males
  - Mean gestational age 39 weeks (sd 2.6)
  - Race:
    - Whites: 48.4%;
    - African American: 2.7%;
    - Native American: 0.5%;
    - Asian or Pacific Islander: 8.2%;
    - Mixed or others: 40.2%
Folate and DNA methylation

Linear regression reveals inverse relationship between folate intake in pregnancy and DNA methylation at birth

McNemar p = 10^{-15}
QQ-plots of observed vs. expected p-values in two sample sets, for folic acid intake.
Top four replicated genes in relation to folate intake

Two of these, *OTX2* and *TFAP2A*, are known genes involved in neural crest development.

*STX11* – associated with lymphocyte/histiocyte overgrowth syndrome.
Are DNA methylation alterations *functional*?

To assess this, compare DNA methylation states to normalized gene expression.
**STX11** gene expression in function of DNA methylation in its promoter (cg22664307) in 86 pluripotent stem cells and their derivatives (data source: GEO, GSE30654).
**OTX2** gene expression in function of DNA methylation in its promoter (cg21039708) in 86 pluripotent stem cells and their derivatives (data source: GEO, GSE30654).
TFAP2A gene expression in function of DNA methylation in its promoter (cg15219145) in 86 pluripotent stem cells and their derivatives (data source: GEO, GSE30654).
Correlation between DNA methylation at 369 bp ahead of the promoter (cg13499966) of **CYS1** and its expression in peripheral mononuclear blood cells of 20 healthy adult men (data source: GEO, GSE49065).
Folic acid is associated with decreased DNA methylation?

Maternal nutrition at conception modulates DNA methylation of human metastable epialleles

Paula Dominguez-Salas¹, Sophie E. Moore¹, Maria S. Baker², Andrew W. Bergen³, Sharon E. Cox¹, Roger A. Dyer⁴, Anthony J. Fulford¹, Yongtao Guan²,⁵, Eleonora Laritsky², Matt J. Silver¹, Gary E. Swan⁶, Steven H. Zeisel⁷, Sheila M. Innis⁴, Robert A. Waterland²,⁵, Andrew M. Prentice¹ & Branwen J. Hennig¹
Replication of Dominguez-Salas CpG sites in CCLS data

Concordant CpG sites
Folate and DNA methylation

Folate is inversely related to DNA methylation in a folate replete population

Several primary targets are related to pediatric developmental syndromes affected by pregnancy folate

Folate’s effects may result in gene expression alterations
Thanks to

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- Todd Whitehead
- Amanda Wheeler
- Steve Francis
- Libby Morimoto
- Patricia Buffler