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

Joseph Wiemels

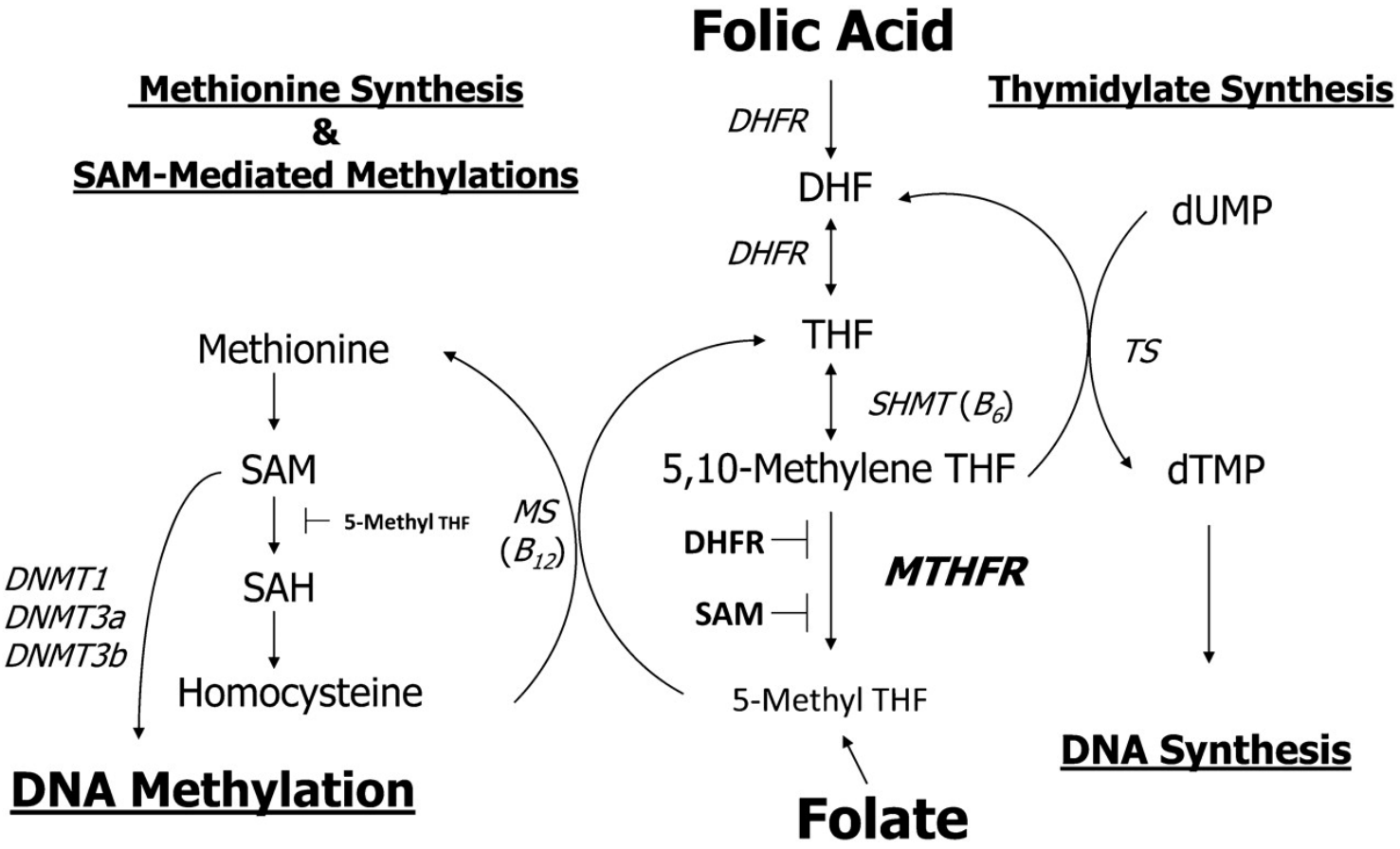
Dept. of Epidemiology & Biostatistics

UCSF
University of California
San Francisco

Outline

- Relationship of folic acid and childhood leukemogenesis
- Folate intake in pregnancy and DNA methylation at birth

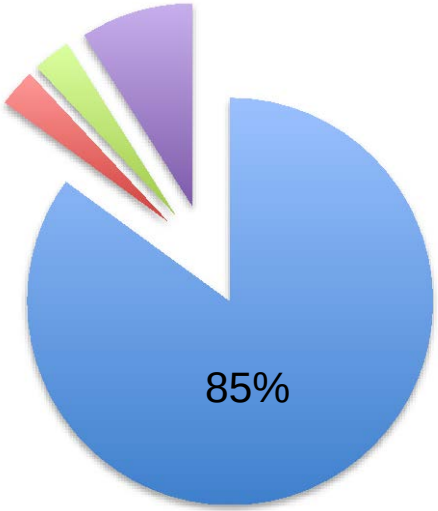
How might folate influence DNA methylation and leukemia?



Subtypes of childhood acute lymphoblastic leukemia

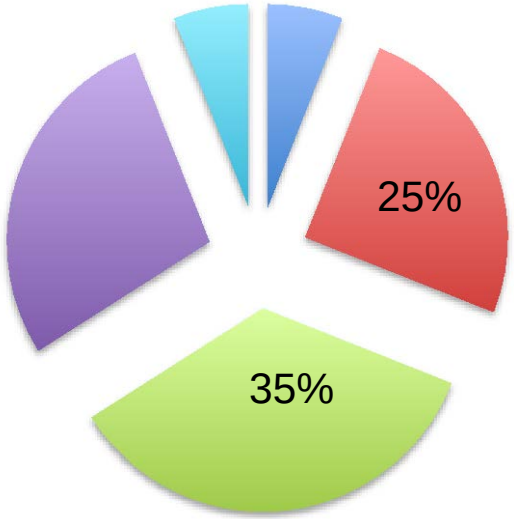
INFANTS

< 1 YR



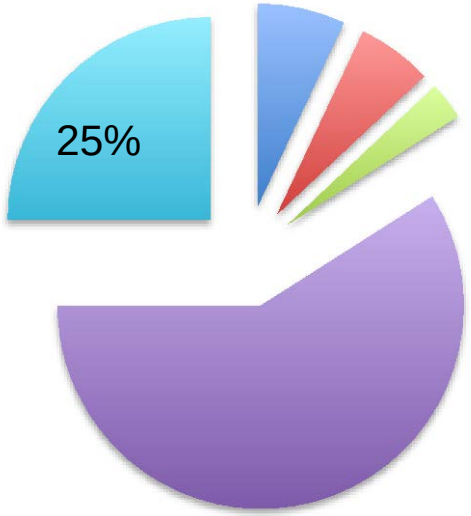
CHILDREN

1 – 15 YRS



ADULTS

16+ YRS



Hyperdiploidy

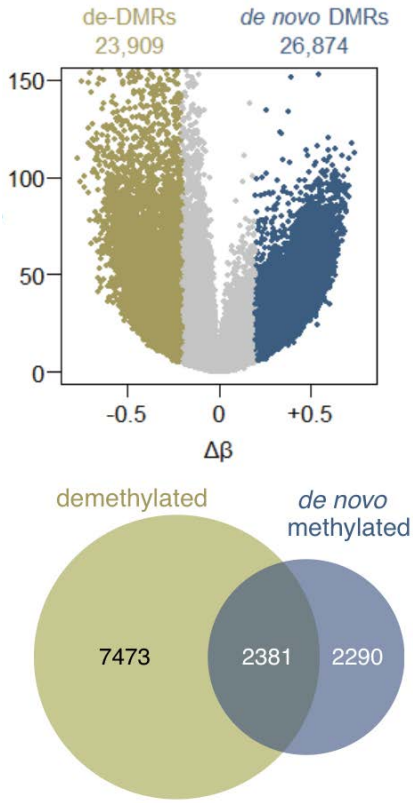
t(12;12) *TEL-AML1*

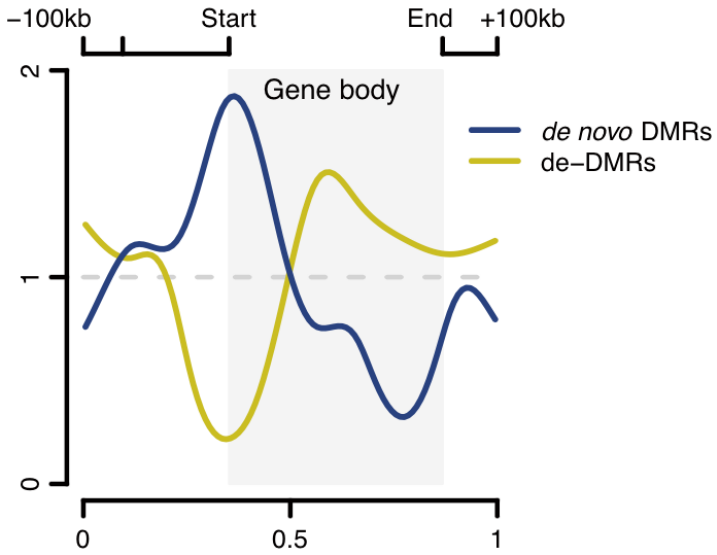
11q23 *MLL*

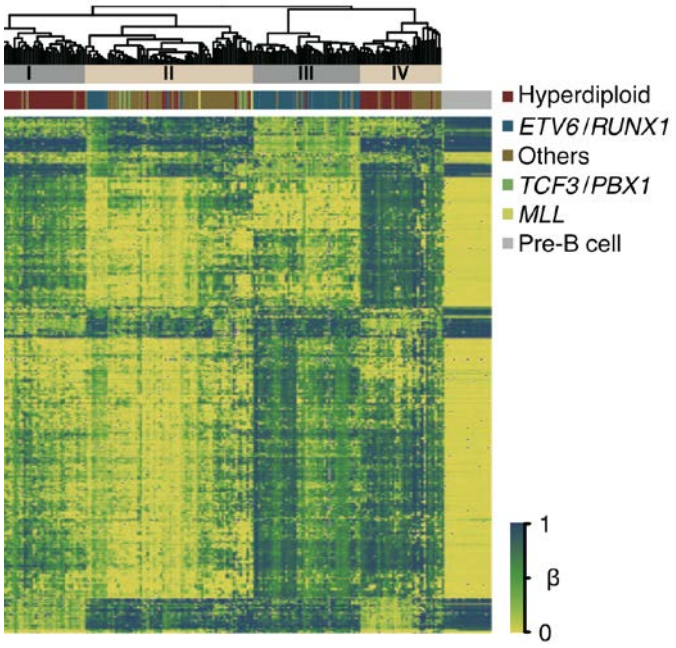
Others

t(9;22) *BCR-ABL1*

Leukemia exhibits profound DNA methylation changes



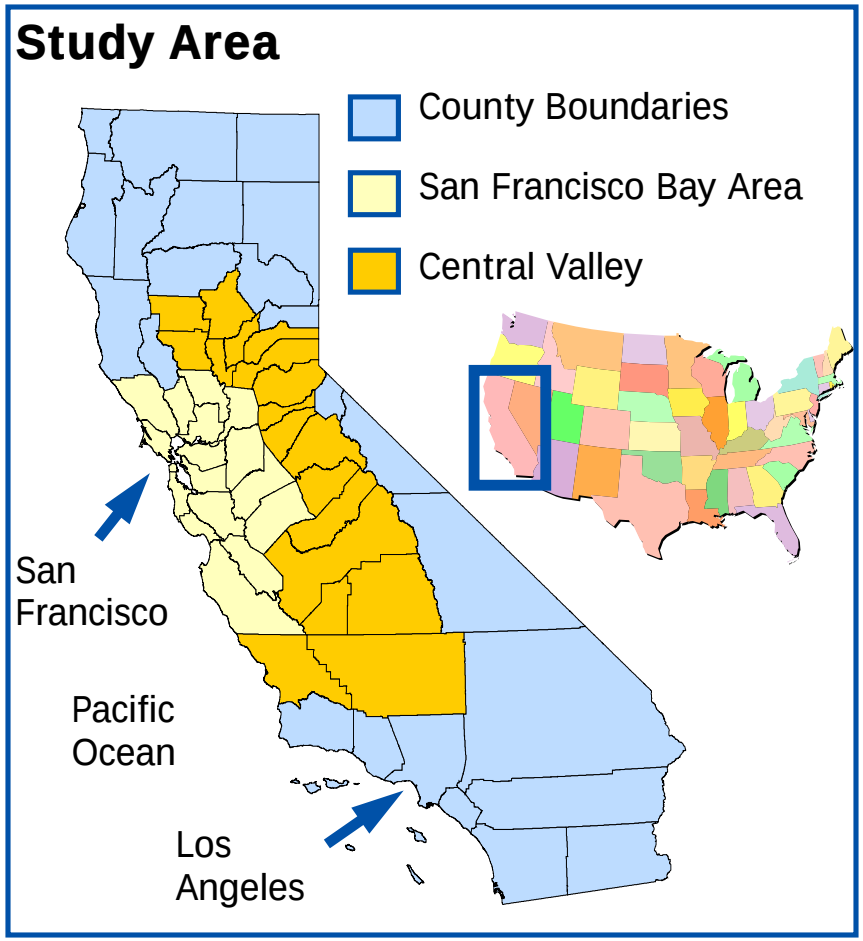




Lee et al, *Nucleic Acids Res*, 2015

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

	Odds Ratio (95% CI)	P-value
Principal Component[a]	0.91 (0.84-0.99)	0.03
Folate (100 DFE/day)	0.97 (0.94-1.01)	0.09
Vitamin B12 (1 µg/day)	0.96 (0.93-0.99)	0.03
Vitamin B6 (1 mg/day)	0.89 (0.79-0.99)	0.05
Riboflavin (1 mg/day)	0.88 (0.77-0.99)	0.05
Methionine (1 g/day)	0.89 (0.73-1.10)	0.28

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.

Childhood Leukemia International Consortium - CLIC

Maternal Supplementation with Folic Acid and Other Vitamins and Risk of Leukemia in Offspring

A Childhood Leukemia International Consortium Study

Catherine Metayer,[a] Elizabeth Milne,[b] John D. Dockerty,[c] Jacqueline Clavel,[d] Maria S. Pombo-de-Oliveira,[e] Catharina Wesseling,[f] Logan G. Spector,[g] Joachim Schüz,[h] Eleni Petridou,[i] Sameera Ezzat,[j] Bruce K. Armstrong,[k] Jérémie Rudant,[d] Sergio Koifman,[l] Peter Kaatsch,[m] Maria Moschovi,[i] Wafaa M. Rashed,[j] Steve Selvin,[a] Kathryn McCauley,[a] Rayjean J. Hung,[n] Alice Y. Kang,[a] and Claire Infante-Rivard[o]

Epidemiology 2014;25: 811–822

Childhood Leukemia International Consortium Pooled Analyses Childhood ALL

	Vitamins (Any Time)						Folic Acid (Any Time)					
	No. Studies	No. Exposed		OR	(95% CI)[a]	Test for Interaction	No. Studies	No. Exposed		OR	(95% CI)[a]	Test for Interaction
		Controls	Cases					Controls	Cases			
Parental education[c]												
Overall[b]	12	6640	4336	0.85	(0.78–0.92)		8	2164	1228	0.80	(0.71–0.89)	
None/Primary	12	873	447	0.72	(0.60–0.88)	$P = 0.14$[d]	8	352	132	0.47	(0.33–0.68)	$P = 0.01$[d]
Secondary	12	2649	1879	0.78	(0.68–0.88)		8	660	410	0.73	(0.59–0.90)	
Tertiary	12	3118	2010	0.97	(0.86–1.09)		8	1152	686	0.96	(0.82–1.12)	

[a]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)

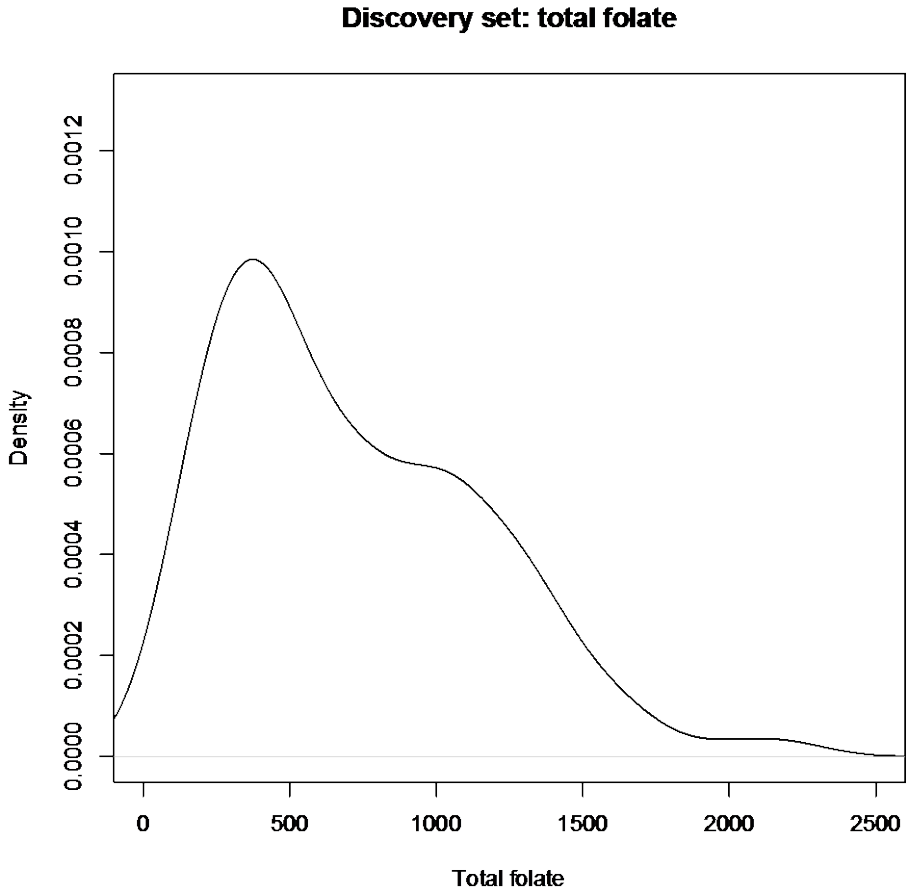
Used to trace back leukemia origin to fetal period

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
  - lm(M-value ~ folate + sex + gestational age + cell-mixture estimates + 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

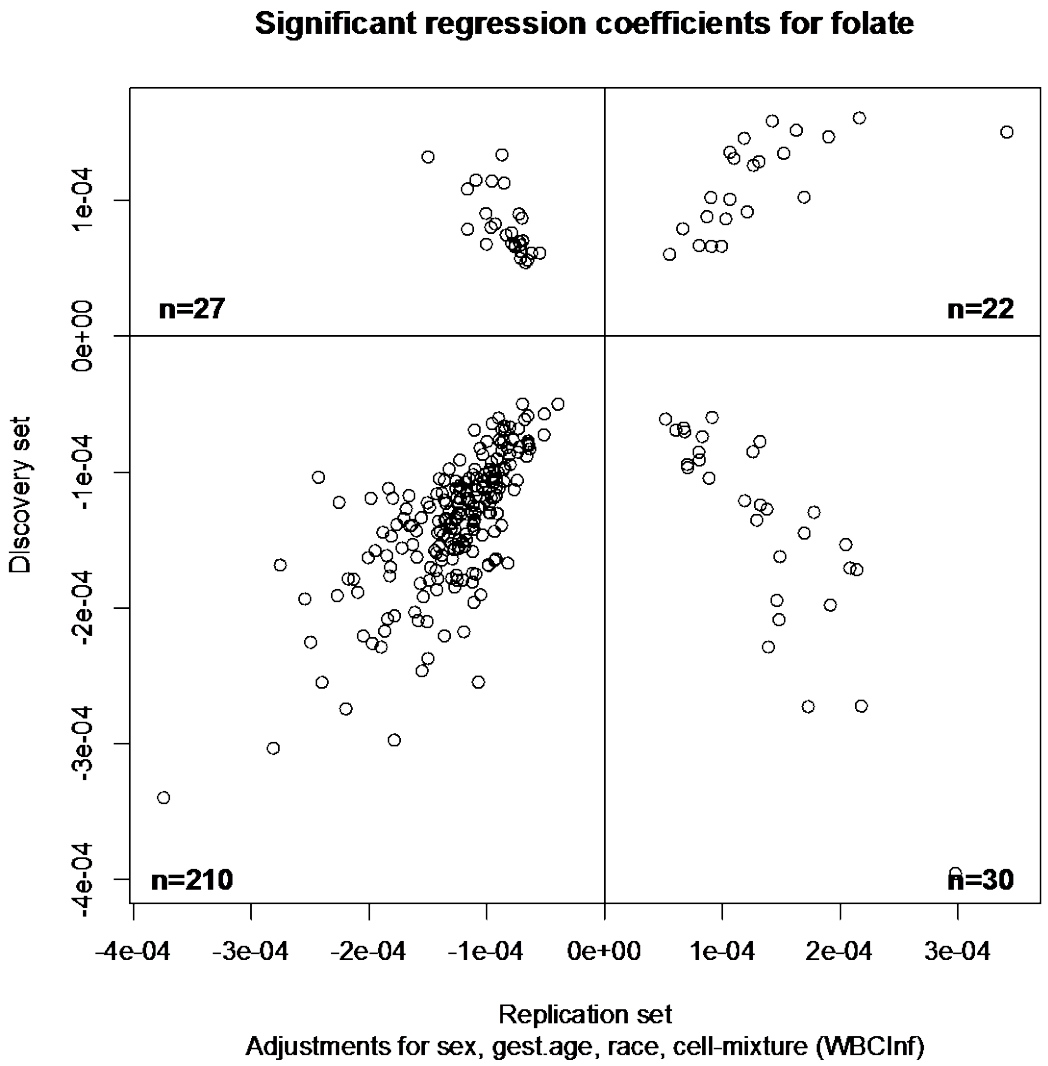
- Clinical characteristics in the discovery set:
  - Folate: range from 54.2 to 2229.0, mean=678.2 and sd=430.4 [mg/d]
  - 57.8% of males
  - Mean gestational age 39 weeks (sd 2.6)
  - Race:
    - Whites: 48.4%;
    - African American: 2.7%;
    - Native American: 0.5%;
    - Asian or Pacific Islander: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



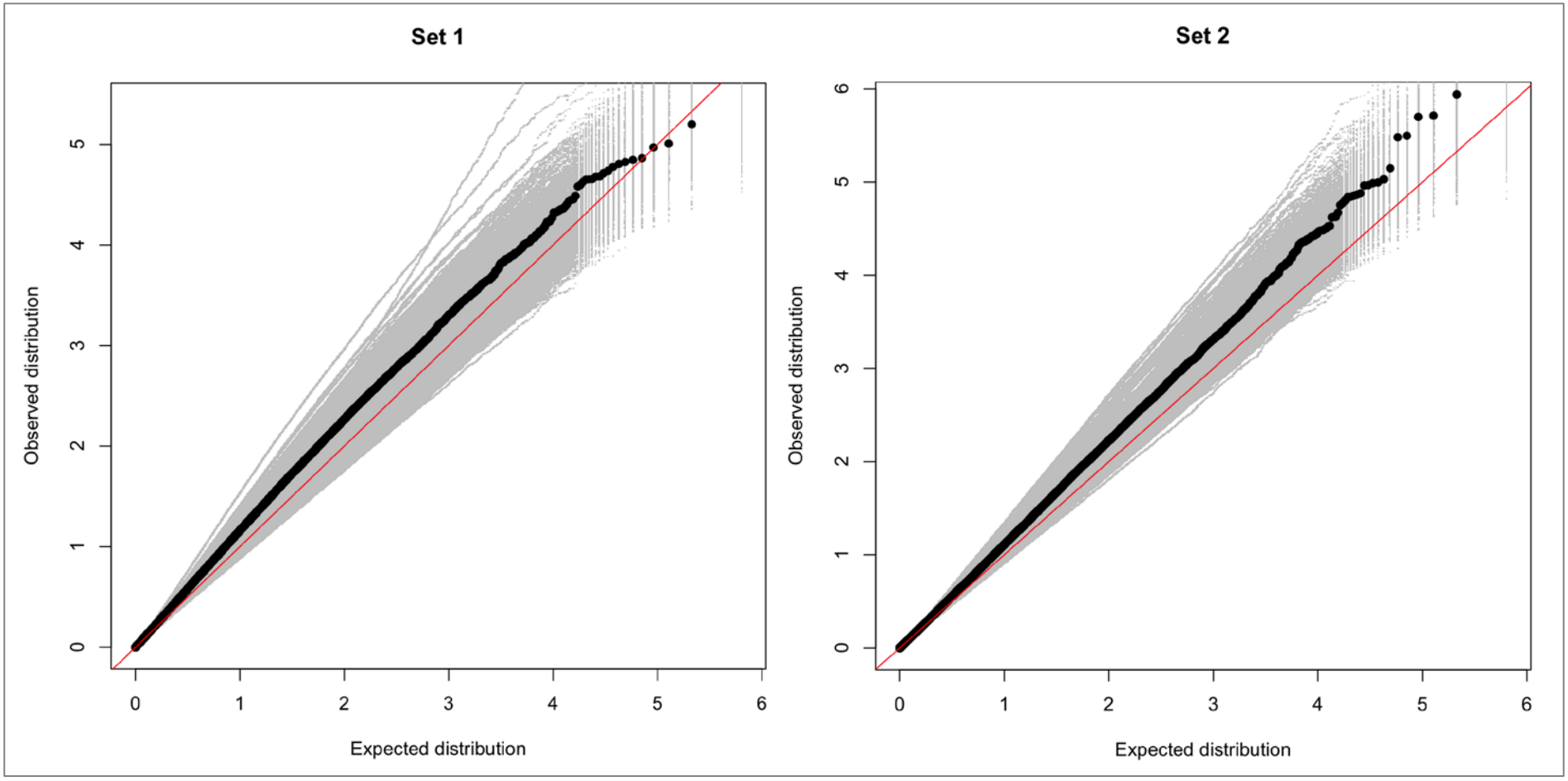
Folate and DNA methylation

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

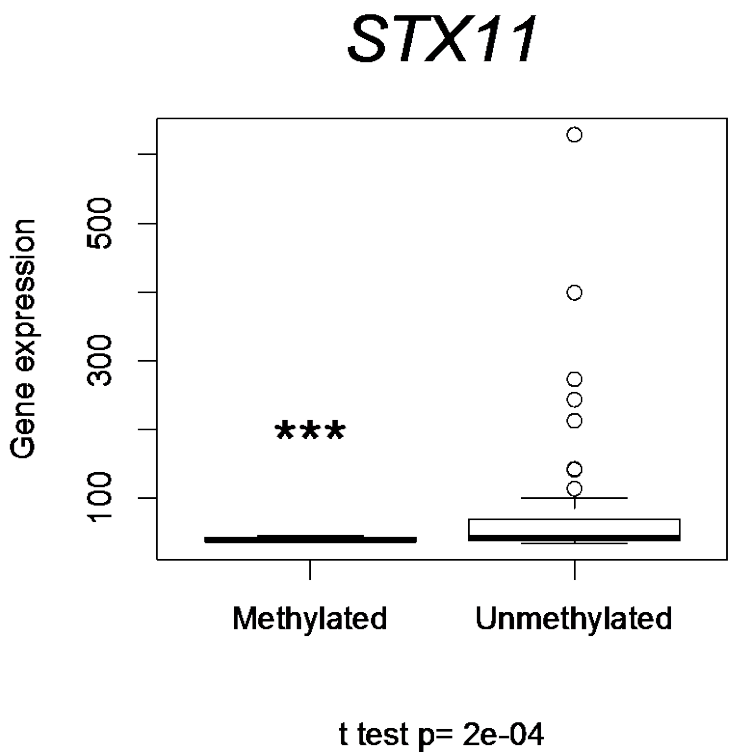
	Set 1					Set 2								
			1,000 bootstrap					1,000 bootstrap						
CpG ID	**Regression coef.**	**p-value**	**Median p-value**	**IQR 25%**	**IQR 75%**	**Regression coef.**	**p-value**	**Median p-value**	**IQR 25%**	**IQR 75%**	**Associated gene**	**Chr.**	**Chr. location**	**CpG location**
cg22664307	-0.00013	0.00054	0.00722	0.002	0.021	-0.00026	0.00099	0.01127	0.003	0.034	*STX11*	6	q24.2	89 bp ahead of the promoter
cg21039708	-0.00021	0.00132	0.01178	0.004	0.038	-0.00034	0.00006	0.00191	0.000	0.008	*OTX2*	14	q22.3	promoter
cg15219145	-0.00016	0.00170	0.01477	0.004	0.043	-0.00023	0.00163	0.01385	0.004	0.041	*TFAP2A*	6	p24.3	promoter
cg13499966	-0.00012	0.00241	0.01666	0.005	0.049	-0.00021	0.00040	0.00622	0.002	0.020	*CYS1*	2	p25.1	369 bp ahead of the promoter

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

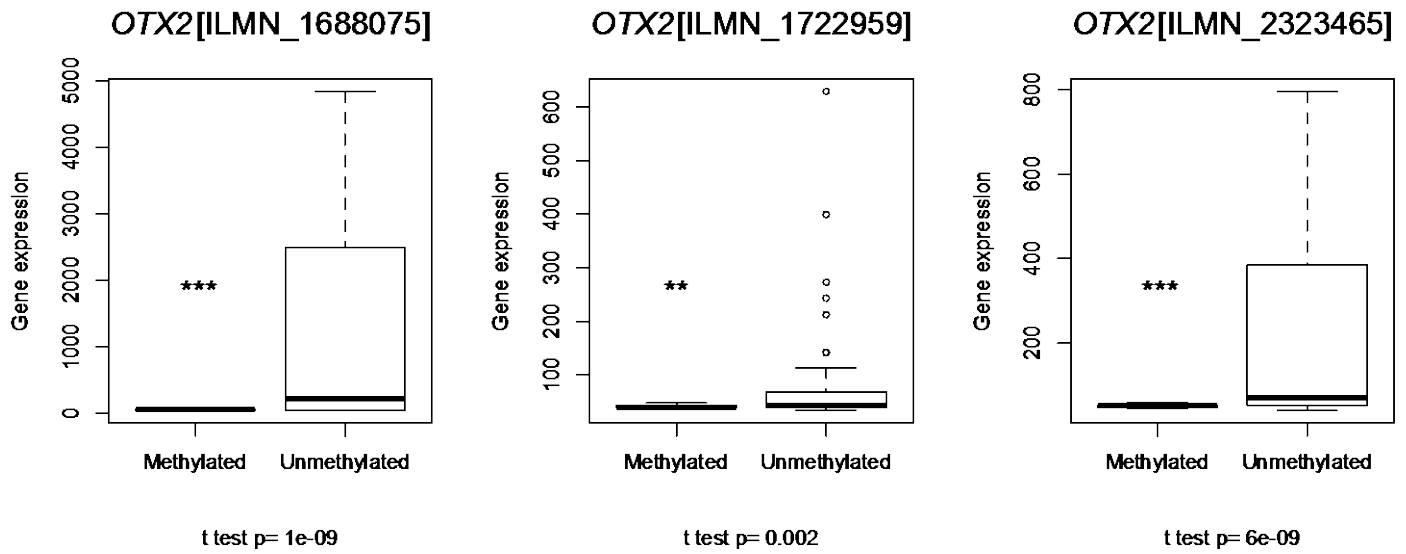
STX11 – associated with lymphocyte/histiocyte overgrowth syndrome

Are DNA methylation alterations *functional?*

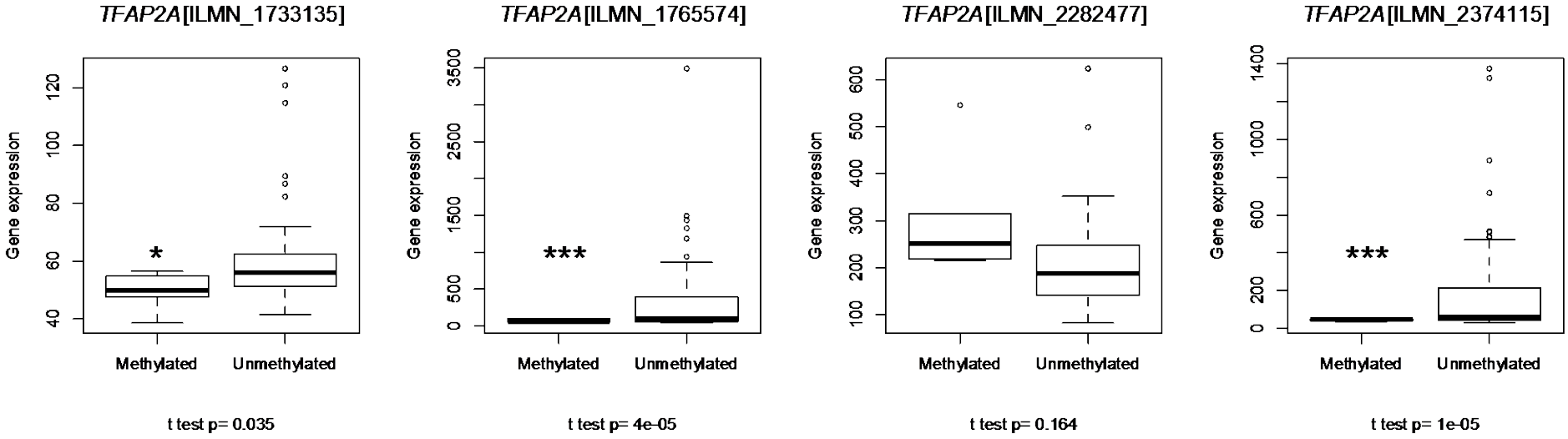
To assess this, compare DNA methylation states to normalized gene expression



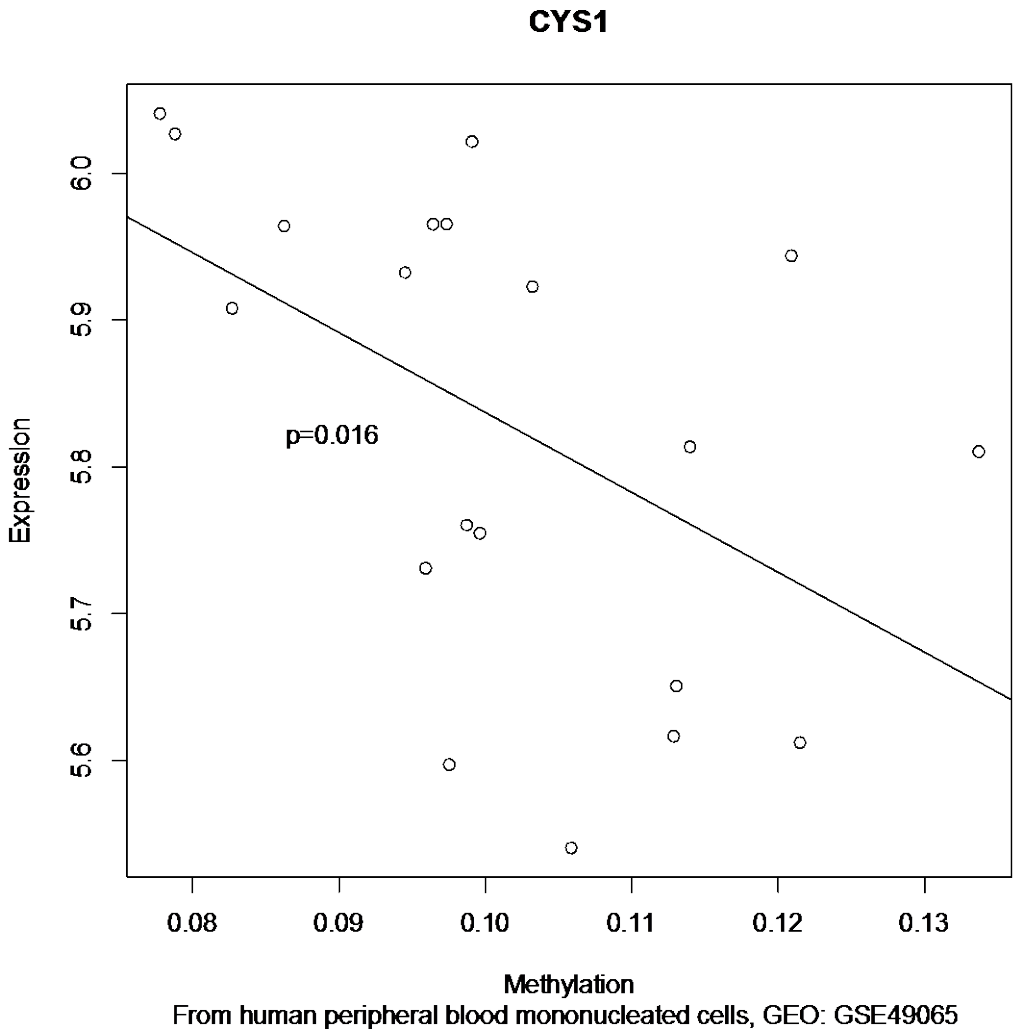
***STX11* gene expression in function of DNA methylation in its promoter** (*cg22664307*) in 86 pluripotent stem cells and their derivatives (data source: GEO, GSE30654).



***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?

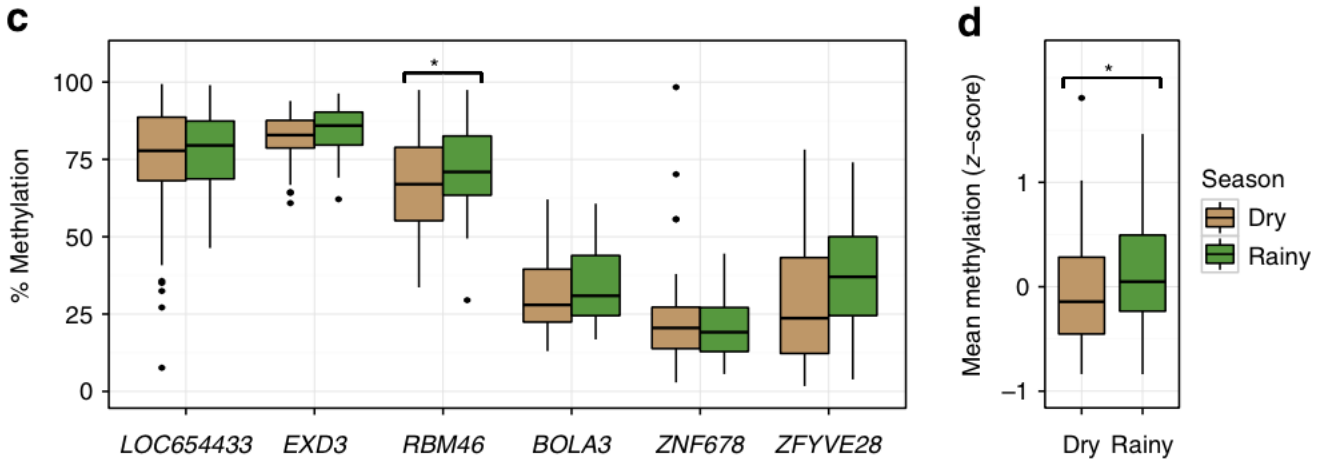
ARTICLE

Received 27 Nov 2013 | Accepted 26 Mar 2014 | Published 29 Apr 2014

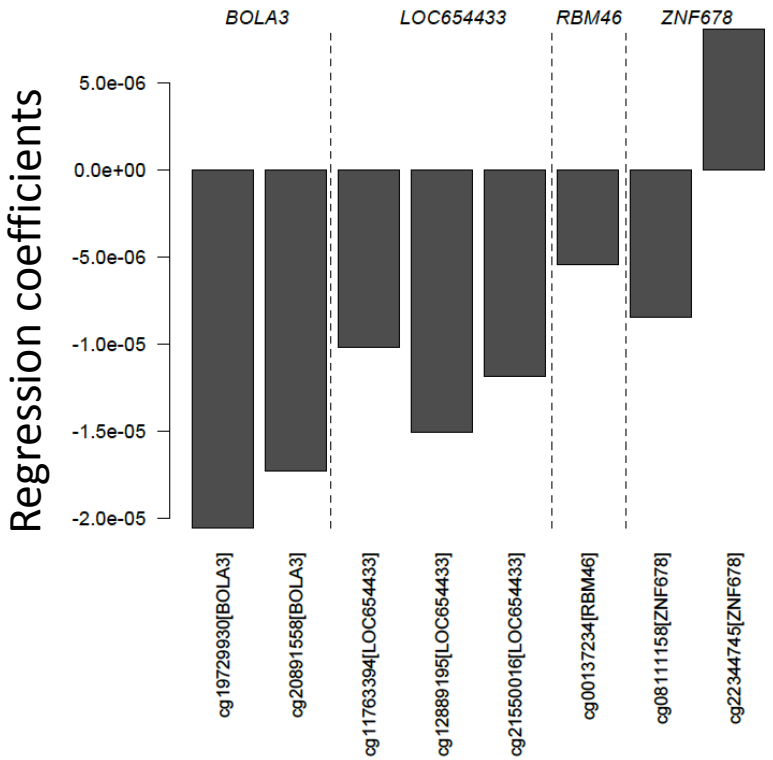
DOI: 10.1038/ncomms4746 OPEN

Maternal nutrition at conception modulates DNA methylation of human metastable epialleles

Paula Dominguez-Salas[1], Sophie E. Moore[1], Maria S. Baker[2], Andrew W. Bergen[3], Sharon E. Cox[1], Roger A. Dyer[4], Anthony J. Fulford[1], Yongtao Guan[2,5], Eleonora Laritsky[2], Matt J. Silver[1], Gary E. Swan[6], Steven H. Zeisel[7], Sheila M. Innis[4], Robert A. Waterland[2,5], Andrew M. Prentice[1] & Branwen J. Hennig[1]



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

Berkeley
UNIVERSITY OF CALIFORNIA

Center for Integrative Research on
Childhood Leukemia
and the Environment

Thanks to

UCSF	UC Berkeley
Semira Gonseth-Nussle Ritu Roy Adam de Smith Kyle Walsh Margaret Wrensch Shweta Choudhry Scott Kogan	Catherine Metayer Todd Whitehead Amanda Wheeler Steve Francis Libby Morimoto Patricia Buffler

UCSF
University of California
San Francisco

CDPH
California Department of
PublicHealth

NIEHS

UNITED STATES
ENVIRONMENTAL PROTECTION AGENCY