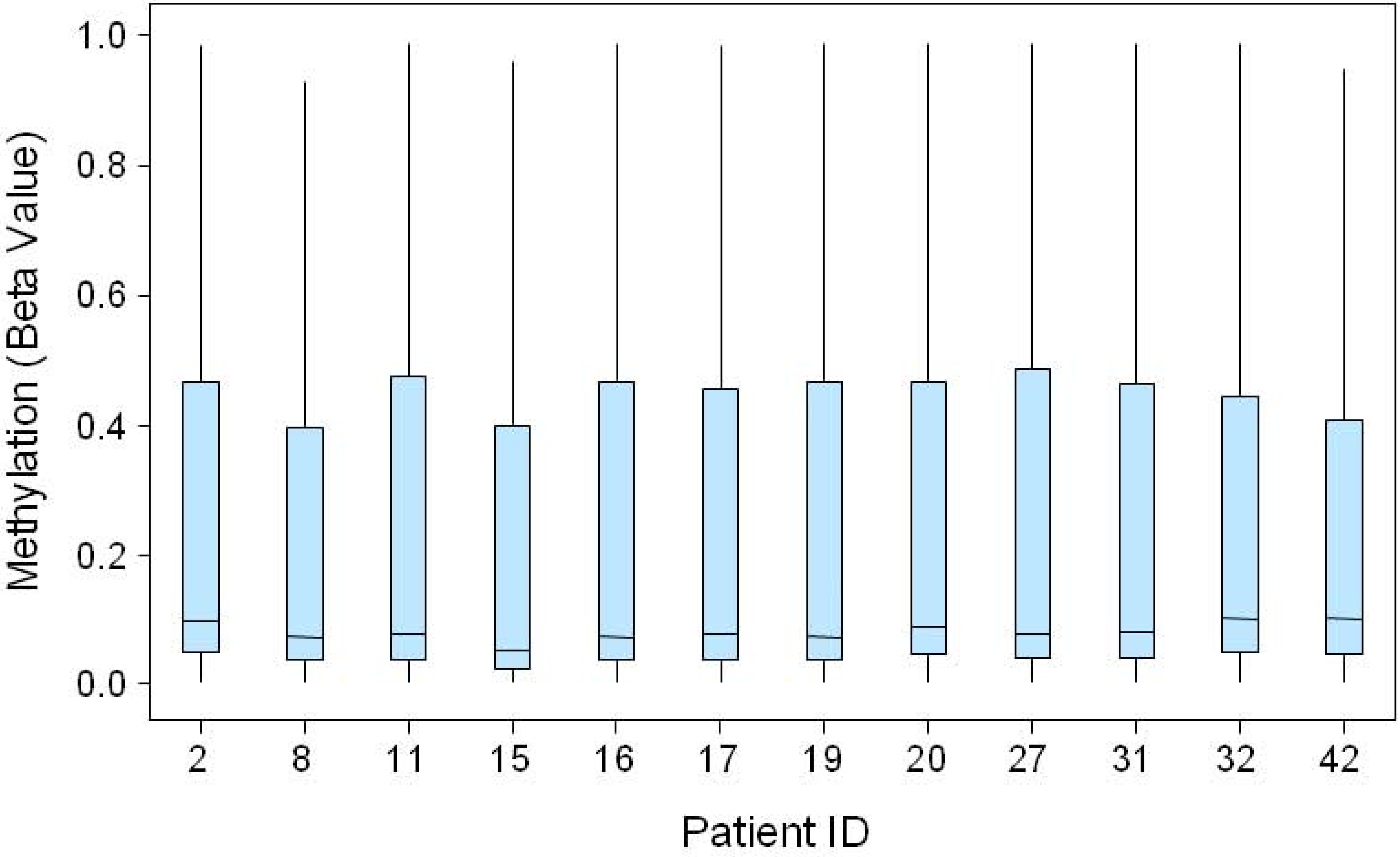
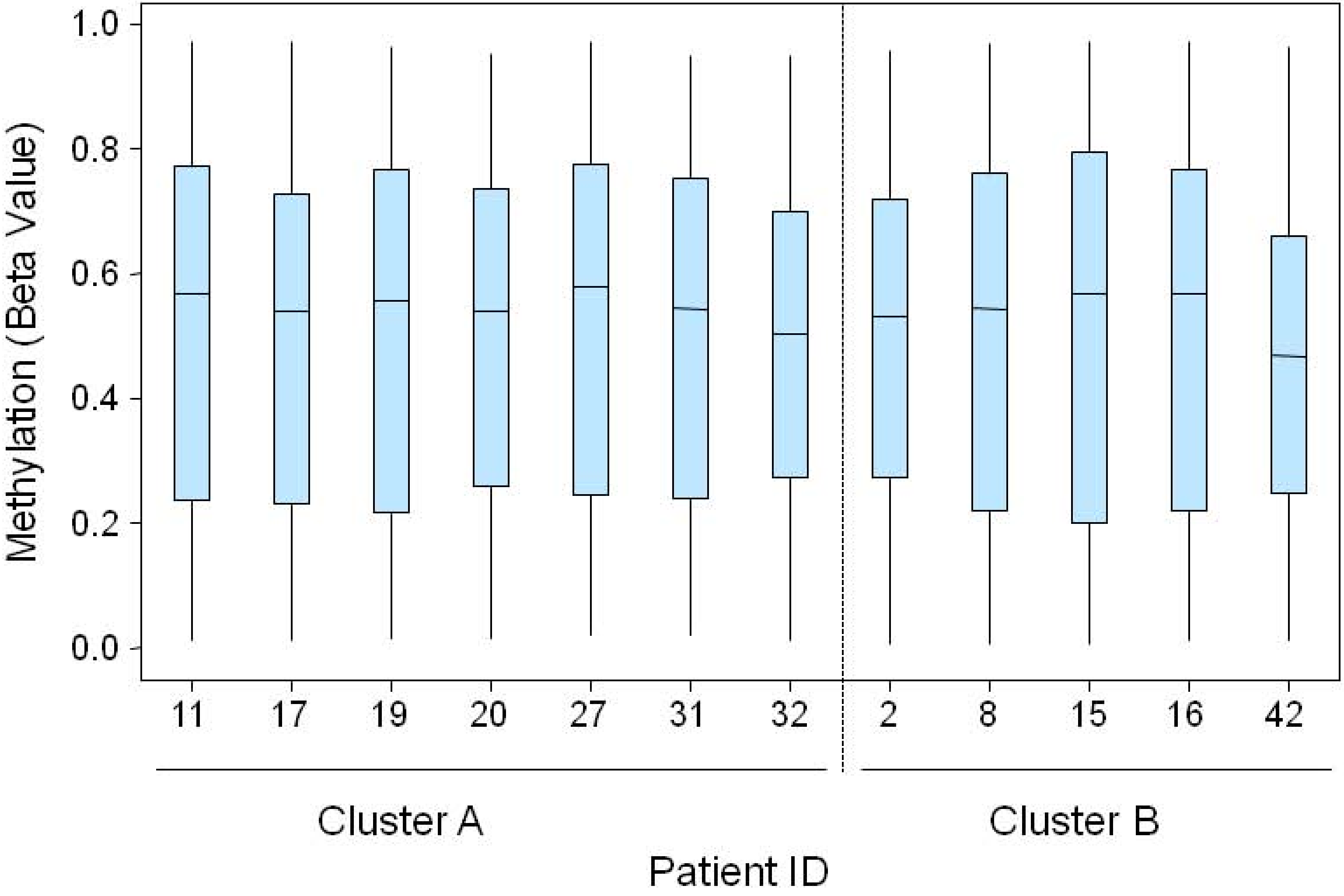
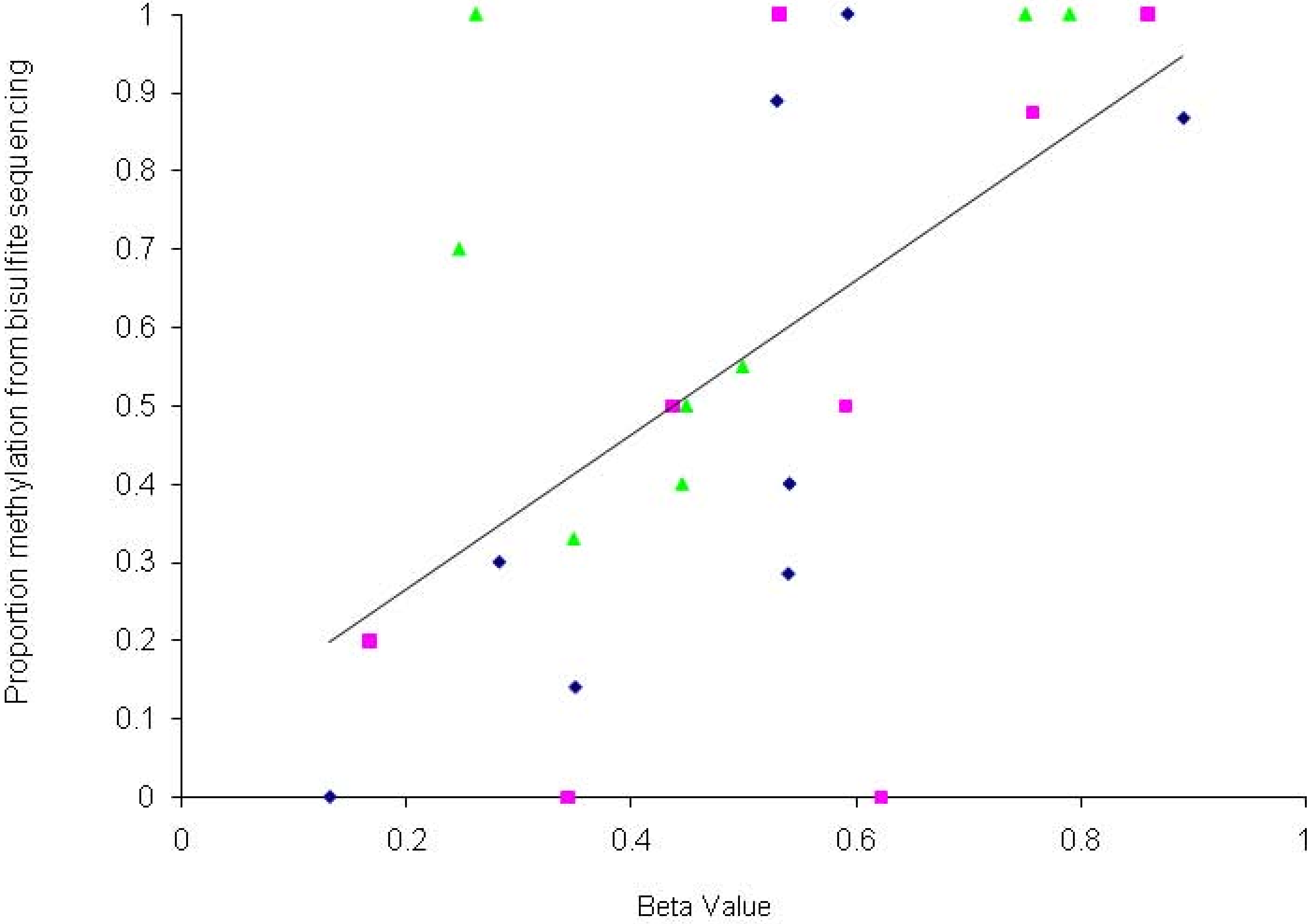
Fryer et al Supplemental Figure S1



Fryer et al Supplemental Figure S2



Fryer et al Supplemental Figure S3



Supplemental Figure S1

Box-whisker plot of methylation values for 21,261 CpGs (detection P value > 0.05 and null values removed) for twelve patient samples. Box represents inter-quartile range (IQR), Horizontal line = median, whiskers extend to 1.5 x IQR.

Supplemental Figure S2

Box-whisker plot of methylation values for clustered 6,5321 CpGs (detection P value > 0.05, null values ad invariant sites removed).Patient samples are grouped by cluster. Box represents inter-quartile range (IQR), Horizontal line = median, whiskers extend to 1.5 x IQR.

Supplemental Figure S3

Bead Array validation. Seven CpG sites with a range of beta values were selected for validation by bisulphite sequencing in three patient samples (11: ♦, 19: ■ and 42: ▲) with an average of 7.5 clones per site per sample. This showed a strong overall positive correlation (as illustrated by trend line) between beta value and proportion of methylated sites from bisulphite sequencing (r = 0.58, p=0.003).

Supplemental Table 1

Relationship between folic acid supplementation and maternal and fetal characteristics

Supplemental Table 2

Genes where methylation is significantly associated with birth weight centile, plasma homocysteine and LINE-1 methylation. Column headings are Symbol; HUGO gene symbol, Chr; chromosome location of CpG measured, MapInfo; base-pair position of C of target CpG, TargetID; unique illumina reference for target CpG, Pt x; Patient identifier numbers, Correlation with LINE-1; correlation coefficient between beta value and LINE-1 methylation, Correlation with Homocysteine; correlation coefficient between beta value and plasma homocysteine, Correlation with Folate; correlation coefficient between beta value and folate, Correlation with Birth weight centile; correlation coefficient between beta value and birth weight centile, significant correlation with LINE-1; significant correlation found between beta value and LINE-1 methylation (yes/no), significant correlation with Homocysteine; significant correlation found between beta value and plasma homocysteine (yes/no), significant correlation with Folate; significant correlation found between beta value and Folate (yes/no), significant correlation with Birth Weight centile; significant correlation found between beta value and birth weight centile (yes/no).

Supplemental Table 3

Identification of functional terms enriched within genes where methylation is significantly associated with birth weight centile, plasma homocysteine and LINE-1 methylation. All enriched categories were determined using the Database for Annotation, Visualization and Integrated Discovery (DAVID) software package. EASE scores < 0.05 were considered significant. Column headings are Category; database queried, Term; definition of term within category, Count, number of genes found in a term, Pvalue (EASE); probability of enrichment as determined by the EASE statistic a modified Fishers exact test, Genes; names of genes found in term, Fold Enrichment; fold enrichment of genes identified in term compared to genome wide membership of that term.

Supplementary Table 1

Folic acid supplementation	No	Yes	*P**
N	4	8	
Maternal age (years)	27 (23-31)	33 (22-37)	0.497
Maternal BMI	31 (22-32)	28 (23-34)	0.819
Parity	0 (0-2)	1 (0-1.5)	0.662
Fetal serum folate	12.6 (10.8-20.0)	16.0 (14.4-20.0)	0.195
Fetal plasma homocysteine (µmol/L)	10.9 (8.2-14.2)	11.0 (7.4-13.6)	0.850
Maternal serum folate	4.7 (4.1-6.1)	7.2 (6.7-13.2)	0.083
Maternal plasma homocysteine (µmol/L)	9.2 (7.5-10.9)	11.4 (9.7-14.2)	0.149

* P value calculated using Mann-Whitney U test