

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to [508 standards](#) due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Supplemental Material

Customized MethylC-Capture Sequencing to Evaluate Variation in the Human Sperm DNA Methylome Representative of Altered Folate Metabolism

Donovan Chan, Xiaojian Shao, Marie-Charlotte Dumargne, Mahmoud Aarabi, Marie-Michelle Simon, Tony Kwan, Janice L. Bailey, Bernard Robaire, Sarah Kimmins, Maria C. San Gabriel, Armand Zini, Clifford Librach, Sergey Moskovtsev, Elin Grundberg, Guillaume Bourque, Tomi Pastinen, and Jacquetta M. Trasler

Table of Contents

Figure S1. Details of human sperm capture panel and comparisons to other commercially available assays. A) Chromosomal ideogram of targeted regions found in the human sperm capture panel. B) Intersection of common CpGs from the different assays. C) Distribution by genomic regions (top) and CpG islands (bottom) of “Intermediate” methylation CpGs (left) and sites covered by Illumina’s Infinium Methylation EPIC (850K, middle) and TruSeq Methyl Capture EPIC (right). Chr, chromosome; TSS, transcriptional start site; TTS, transcriptional termination site; UTR, untranslated region.

Figure S2. Analysis of EPIC related content not targeted by the human sperm capture design. Sites not targeted by the human sperm capture panel but found on the Infinium Methylation EPIC array (left) or the TruSeq Methyl Capture EPIC (right). Depiction of the untargeted CpG distributed within repetitive elements (top) and CpG islands/shores/shelves (bottom). LINE, long interspersed nuclear elements; SINE, Short interspersed nuclear elements; LTR, Long terminal repeats.

Figure S3. Analysis of DNA methylation variation of different CpG sites captured by the human sperm capture panel. The standard deviation of DNA methylation, of individual sites captured by our human sperm panel, was calculated from libraries (a minimum of 20x coverage, sequenced in at least 30 of 45 individuals). The average variation from “Intermediate” methylation (n=571,584) and 850K derived (n=1,040,764) sites was determined and plotted. Similarly, the average variation from five different sets of randomly chosen 850K derived sites (R1-R5 850K), where a similar number as “Intermediate” methylation sites was selected, was also plotted. Mean + SD; Unpaired t-test between “Intermediate” and 850K, **** p<0.001; ANOVA between “Intermediate ” and random (R1-R5 850K) data, **** p<0.001.

Figure S4. Frequency of coverage (under 100-fold) of A) off-target and B) on-target CpG sites. C) Effect of coverage on correlation and DNA methylation between data obtained by WGBS and Capture sequencing of a same pooled DNA sample. D) Principle component analysis of all samples captured with the human sperm capture panel, color coded by *MTHFR* genotype. E) Table summarizing the average total CpGs sequenced, DNA methylation and coverage of all sequenced CpGs and those specifically captured by the capture panel (on-target). Average calculated from MCC-Seq of individual subjects (n=45) from the Toronto (21 participants) and Montreal (12 participants x 2 time points). Mean +/- SEM. *MTHFR*, methylenetetrahydrofolate reductase; PC, principle component.

Figure S5. Examination of imprinted gene methylation from the human sperm capture panel data. Sperm DNA methylation of paternally and maternally methylated imprinted genes in A) a cohort of fertile men from Toronto (n=13 *MTHFR* 677CC and n=8 677TT) and B) from an infertile cohort of men from Montreal (n=6 per genotype and time point), before and after the use of high dose folic acid supplementation (5mg/day). Mean +/- SEM. *MTHFR*, methylenetetrahydrofolate reductase.

Figure S6. Effect of *MTHFR* genotype on sperm DNA methylation. A) Q-Q and Manhattan plots of association p-values when comparing *MTHFR* 677CC vs. 677TT genotypes. In a mouse model, the effect of *Mthfr* heterozygosity on sperm DNA methylation (data from Aarabi et al. 2018) demonstrated B) genomic region distribution of DMCs, C) increases in sperm DNA methylation and D) magnitude methylation of differences similar to those of 677TT subjects (Figure 5). *MTHFR/Mthfr*, methylenetetrahydrofolate reductase; DMCs, differentially methylated cytosines; TSS, transcriptional start site; TTS, transcriptional termination site; UTR, untranslated region.

Figure S7. Individual participant validation of DMCs between *MTHFR* 677CC and 677TT individuals. Pyrosequencing data (n=5 per *MTHFR* genotype) is overlaid with their associated human sperm capture panel data for validation of two loci: A) an intron of *SAMD11* and B) intergenic region. X-axis: chromosomal base pair location; Y-axis: % DNA methylation. chr, chromosome; CC, *MTHFR* 677CC; TT, *MTHFR* 677TT; *MTHFR*, methylenetetrahydrofolate reductase.

Figure S8. Measurement of serum and RBC folate in individuals before and after 6-month folic acid (5mg/day) supplementation. A) Serum and B) red blood cell (RBC) folate levels measured in *MTHFR* 677CC and 677TT participants (n=6 per genotype), before and after a 6 month treatment with high dose folic acid supplements (5mg/day). Mean +/- SEM; Paired t-test, * p<0.05, ** p<0.01. *MTHFR*, methylenetetrahydrofolate reductase.

Figure S9. Effect of high-dose folic acid on the human sperm DNA methylome. Q-Q and Manhattan plots of association p-values when comparing the effect of high-dose folic acid supplementation in infertile A) *MTHFR* 677CC and B) 677TT subjects. C) The re-analysis of the RRBS data (Aarabi, M. 2015) using generalized linear regression models to identify differentially methylated cytosines. Gene Ontology analysis for enrichments of biological processes was performed with DMCs within genic regions from subjects of D) 677CC and E) 677TT genotypes. *MTHFR*, methylenetetrahydrofolate reductase; RRBS, reduced representation bisulfite sequencing; DMCs, differentially methylated cytosines.

Figure S10. Analysis of folate metabolism-related DMCs with publically available datasets.

All CpG sites targeted by our human sperm capture panel, as well as DMCs found to be altered due to *MTHFR* genotype and by high-dose folic acid supplementation (in both *MTHFR* 677CC and 677TT groups) were overlapped with functional or sensitive areas in the human genome. The percentage enrichment for sites found overlapping with histone retention sites of A) H3K4me1, B) H3K4me3, C) H3K27ac, and D) H3K27me3, as well as with E) evolutionarily conserved elements, F) conserved sperm DNA methylation patterns and G) sites of altered methylation due to dioxin exposures were analysed. H) The overlap between DMCs discovered through the different folate metabolism exposures was also assessed. *MTHFR*, methylenetetrahydrofolate reductase; DMCs, differentially methylated cytosines.

Additional File- Excel Document

REFERENCES