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Supplemental Material

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

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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 *MTFHR* 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) *MTHFTR 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. <u>Analysis of folate metabolism-related DMCs with publically available datasets</u>. 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