Reviewer's report

Title: Folate network genetic variation, plasma homocysteine, and global genomic methylation content: a genetic association study

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Reviewer: Barry Shane

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This study uses a candidate gene approach to investigate the effect of sequence variation in one carbon metabolism genes on homocysteine levels and DNA methylation in non Hispanic white males in the NAS population. Regression models were used to adjust for age, smoking, and for nutrient status (folate, B6 and B12). Several novel associations between variants in one carbon metabolism genes and homocysteine levels and Alu and LINE-1 methylation are suggested by the data as well as some nutrient gene interactions. Using a FDR adjusted p value of about 0.001 (presumably a Bonferroni adjustment of p< 0.05 and 52 genes), several of these gene associations survive adjustment. The data are interesting and suggest several new avenues to explore.

The manuscript indicates that 330 SNPs in 52 genes were successfully genotyped and studied in relation to plasma homocysteine and global genomic methylation. It also states that a subset of 52 non-redundant SNPs was selected to represent the most likely functional variant with the highest MAF that could be selected for each of the 52 genes. Presumably this was done to limit the Bonferroni hit.

Similarly, the supplementary material states that additive, dominant, recessive, and overdominant genetic models of inheritance were tested for each SNP, and the model yielding the most statistically significant result in the unadjusted single SNP analysis was chosen as the best model going forward. It is not clear whether this refers to the 330 or 52 SNPs and whether this would entail an additional Bonferroni correction. If this involved analysis of the whole data set (homocysteine and methylation) for all 330 SNPs, the adjusted p value should be lower.

The criteria for selecting the SNP chosen for each gene going forward and how the adjusted p values based on the FDR was obtained should be clarified further.

It is somewhat surprising that the MTHFR rs1801133 variant on chromosome 1 (Manhattan plot – fig. 1) did not apparently reach statistical significance in relationship to homcysteine levels, as homocysteine levels were measured prior to folate fortification. This should be commented on more explicitly in the discussion on factors that affect homocysteine levels.

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests.