Genetic and Epigenetic Signature Identifies Individuals with Elevated Response to Vitamin B12 Supplementation

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Abstract

Vitamin B12 is essential for many aspects of human health. Deficiencies are largely caused by inadequate intake or reduced absorption, and are associated with increased incidence of neurological, cognitive and cardiovascular decline [1]. Using a previously published dataset of B12 and folate levels in elderly individuals (GSE74548), we investigated the effect of a variant in the *methylene tetrahydrofolate reductase* (MTHFR) rs1801133 genotype and supplementation status on B12 levels (A) and folate (B). After supplementation, serum B12 levels are significantly different by genotype (P = 0.0073 and 0.47, respectively). MTHFR CC individuals cluster into 2 distinct groups, that may represent high and low responders to supplementation (P = 2.8 x 10^-4). High responders (light blue dots) are significantly different from the rs1801133 TT individuals (black dots; P = 6.9 x 10^-5), as opposed to low responders (dark blue dots; P = 1). P-values were generated using Wilcoxon Rank sum test and vitamin B12/folate serum values.

### Methods

- **B - Vitamins for the PRevention of Osteoporotic Fractures**
- Randomized, placebo-controlled trial (GSE74548) [2]
- Two year daily supplementation with 400ug folic acid/500ug vitamin B12 (N=44) or placebo (N=43)
- DNA for genome-wide DNA methylation (Infinium 450K Bead Chip) and serum for folate acid/ vitamin B12 collected at baseline and 2 year follow-up from buffy coats
- Correlations between quantile transformed serum folate acid/ vitamin B12 levels and DNA methylation at baseline (N=8,036 CpG sites) were determined using standard linear regression in R v3.3.1

### Results

**Figure 1.** Serum vitamin B12, but not folate levels vary by rs1801133 genotype after supplementation

**Figure 2.** Baseline DNA methylation levels are correlated with supplementation induced changes in serum vitamin B12

- Density plot of Spearman correlation p-value distributions for rs1801133 CC (blue line) and TT individuals (dashed line). rs1801133 CC individuals are enriched for low p-values (P < 0.01) (empirical P < 1 x 10^-4). rs1801133 TT individuals are modestly enriched for low p-values (empirical P=0.044). Among rs1801133 CC individuals, baseline serum vitamin B12 levels are modestly elevated in high responders relative to low responders (P=0.13). Scatterplot of median DNA methylation foldchange (baseline - follow-up) for rs1801133 CC individuals receiving placebo (y-axis) or supplementation (x-axis). Red dots represent CpG sites with a P < 0.01 in our analysis.

**Figure 3.** Genes near correlated CpG sites (p < 0.01) are enriched in inflammatory and immunomodulatory pathways

- Genes near correlated CpG sites are significantly enriched in two Ingenuity Pathway Analysis protein-protein interaction networks. A. The first network (score = 46) is centered around the major inflammatory transcription factor, NFkB. B. The second network (score = 43) is centered around many interacting immunomodulatory molecules. ITGB1, CD29, for example, has been linked to enhanced immune cell ability to respond to recall antigens and home to sites of infection [3]. Molecules shaded in grey represent genes that are near a CpG site in which baseline DNA methylation levels are correlated with supplementation induced changes in serum B12 (P < 0.01).

### Conclusions

- Genetic and baseline epigenetic signatures detectable in blood are correlated with and may be able to predict response to vitamin B12 supplementation
- This baseline epigenetic signature may alter the ability of immune cells to respond to inflammatory or immunomodulatory stimuli

### References

