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

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Abstract

Vitamin B_{12} 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 B_{12}/ folic acid supplementation in elderly individuals (GSE74548), we investigated the effect of a variant in the *methylenetetrahydrofolate reductase* gene (*MTHFR*; rs1801133) on serum B_{12} and folate levels at baseline and after 2 years of supplementation. As well as, the association between baseline genome-wide DNA methylation (Infinium 450K DNA methylation chip) in peripheral blood mononuclear cells (PBMCs) and supplementation induced changes in circulating B vitamin levels. Among post-supplementation individuals, we observed that those with the rs1801133 CC genotype had elevated circulating B_{12} but not folic acid levels, relative to the rs1801133 TT genotype (P = 0.0073 and P = 0.47, respectively). Moreover, individuals with the CC genotype clustered into two distinct B_{12} response groups (P = 2.8 $x10^{-6}$), termed high and low responders. Additionally, we observed an enrichment of low p-values among baseline DNA methylation patterns and change in circulating B_{12} levels after supplementation in CC individuals only (empirical P < $1x10^{-5}$). This epigenetic signature is not near genetic variants or genes known to influence vitamin B_{12} uptake. Overall, we present evidence that epigenetic signatures at baseline may predict an individual's response to B_{12} supplementation and may highlight novel pathways of regulation. Elucidation and further understanding of pathways associated with elevated response to B_{12} supplementation, may aid in the treatment of chronically deficient individuals.

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 folic acid/ vitamin B12 collected at baseline and 2 year follow-up from buffy coats
- Correlations between quantile transformed serum folic 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

Figure 1. Serum vitamin B_{12}, but not folate levels vary by rs1801133 genotype after supplementation

Stratification of serum B_{12} levels by *methylene tetrahydrofolate reductase* (*MTHFR*) rs1801133 genotype and supplementation status for serum B_{12} levels (**A**) and folate (**B**). After supplementation, serum B_{12}, not folate, 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 = $2.8x10^{-6}$). High responders (light blue dots) are significantly different from the rs1801133 TT individuals (black dots; P = $6.9x10^{-7}$), as opposed to low responders (dark blue dots; P=1). P-values were generated using Wilcoxon Rank sum test and vitamin B_{12}/folate serum values.

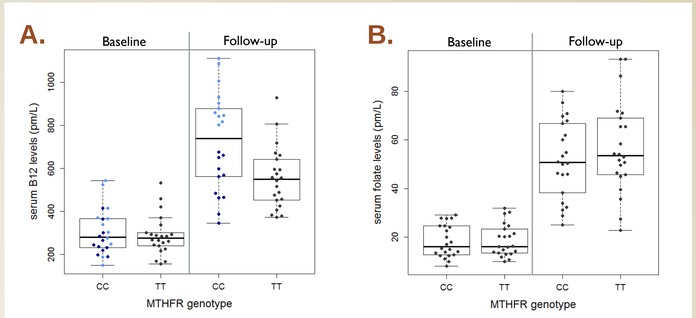


Figure 2. Baseline DNA methylation levels are correlated with supplementation induced changes in serum vitamin B_{12}

A. 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 $x10^{-5}$). rs1801133 TT individuals are modestly enriched for low p-values (empirical P=0.044). **B.** Among rs1801133 CC individuals, baseline serum vitamin B_{12} levels are modestly elevated in high responders relative to low responders (P=0.13). **C.** 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.

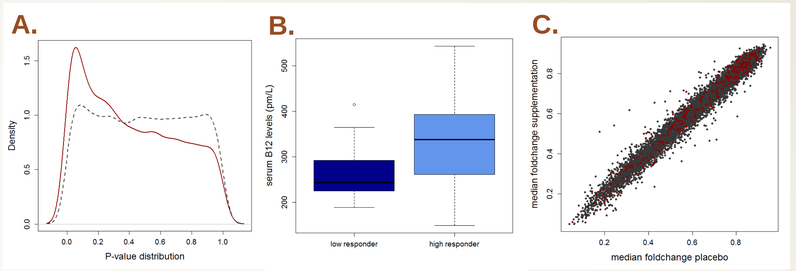
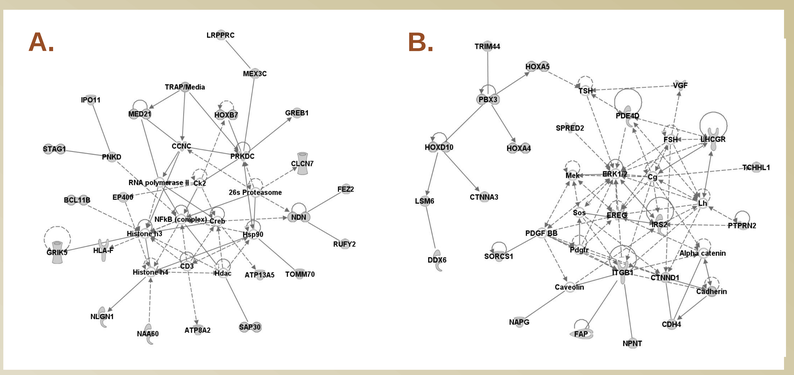


Figure 3. Genes near correlated CpG sites (p < 0.01) are enriched in inflammatory and immunomodulatory pathways

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 B_{12} (P < 0.01).



Conclusions

- Genetic and baseline epigenetic signatures detectable in blood are correlated with and may be able to predict response to vitamin B_{12} supplementation
- This baseline epigenetic signature may alter the ability of immune cells to respond to inflammatory or immunomodulatory stimuli

References

1. Porter, K., et al., *Causes, Consequences and Public Health Implications of Low B-Vitamin Status in Ageing.* Nutrients, 2016. **8**(11).

2. Kok, D.E., et al., *The effects of long-term daily folic acid and vitamin B12 supplementation on genome-wide DNA methylation in elderly subjects.* Clin Epigenetics, 2015. **7**: p. 121.

3. Morimoto, C., et al., *The isolation and characterization of the human suppressor inducer T cell subset.* J Immunol, 1985. **134**(3): p. 1508-15.