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Common variants of *FUT2* are associated with plasma vitamin B₁₂ levels

Aditi Hazra^{1,2}, Peter Kraft¹, Jacob Selhub³, Edward L. Giovannucci^{2,4}, Gilles Thomas⁵, Robert N. Hoover⁵, Stephen J. Chanock⁵, and David J. Hunter^{1,2,4,5}

¹Program in Molecular and Genetic Epidemiology, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA.

²Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA.

³Vitamin Metabolism and Aging Laboratory, Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111, USA.

⁴Department of Nutrition, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA.

⁵Division of Cancer Epidemiology and Genetics, National Cancer Institute(NCI), US National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Bethesda, Maryland 20892, USA.

Abstract

We identified a strong association ($p=5.36\times 10^{-17}$) between rs492602 in *FUT2* and plasma vitamin B₁₂ levels in a genome-wide scan ($n=1,658$) and an independent replication sample ($n=1,059$) from the Nurses' Health Study. Women homozygous for the rs492602 G allele had higher B₁₂ levels. This allele is in strong linkage disequilibrium with the *FUT2* W143X nonsecretor variant, suggesting a plausible mechanism for altered B₁₂ absorption and plasma levels.

Keywords

one-carbon metabolism; plasma vitamin B₁₂; genome-wide association; *FUT2*; secretor locus

Plasma levels of vitamin B₁₂ are modifiable quantitative traits associated with certain diseases¹. Vitamin B₁₂ found in meat (such as liver, shellfish, fish, poultry, and eggs) and milk products² is composed of corrin and cobalt rings and is necessary for the formation of red blood cells, DNA synthesis during cell division, and maintenance of the myelin nerve sheath. Deficiency in vitamin B₁₂, clinically associated with pernicious anemia, cardiovascular disease, cancer, and neurodegenerative disorders, is often related to poor intestinal B₁₂ absorption² rather than direct dietary deficiency (the recommended adult intake for vitamin B₁₂ is 2.4 µg/day). In the Nurses' Health Study (NHS), we previously observed that women in the lowest quartile of plasma vitamin B₁₂ levels had marginally worse cognitive performance (based on a global score averaging 6 cognitive tests) than women in the highest quartile of plasma vitamin B₁₂ and that combined folate and vitamin B₁₂ deficiency was associated with the lowest cognitive performance³.

Epidemiologic studies provide evidence for the association of genes and metabolites in the B-vitamin-mediated plasma one-carbon metabolic pathway with chronic diseases^{2–4}. Rare high penetrance mutations in genes in this pathway affect the ability to digest, absorb⁵, and utilize vitamin B₁₂². However, common genetic variants in candidate genes have not been consistently associated with plasma vitamin B₁₂ levels. Therefore, we conducted a genome-wide association study (GWAS) to identify novel loci that influence plasma vitamin B₁₂ levels in 1,658 women genotyped with the HumanHap500 as part of the Cancer Genetic Markers of Susceptibility Project (CGEMS; Supplementary Methods). Participants were of self-reported European ancestry⁶. Detailed methods have been previously reported, including quality control assessment of genotypes with sample completion and SNP call rates, concordance rate, deviation from Hardy–Weinberg proportions in control DNA, and final sample selection for association analysis of the NHS CGEMS population⁶.

We tested association between each of the 528,134 SNP markers that passed quality control filters and log-transformed plasma vitamin B₁₂ using linear regression, adjusted for age, total methyl intake (defined as total folate, dietary methionine, and alcohol intake), and assay batch. We saw no evidence for systematic bias in the distribution of p-values for analyses with and without further adjustment for residual population structure using the top four principal components of genetic variation (Supplementary Fig. 1), compatible with no confounding of SNP-metabolite associations due to population stratification.

In the initial GWAS, the SNPs on chromosome 19p13.3 accounted for the excess of p-values $<10^{-7}$ (Supplementary Fig. 1: Quantile-Quantile plot). The strongest association with plasma vitamin B₁₂ was rs602662 ($P_{\text{trend}} = 6.54 \times 10^{-10}$; Fig. 1, Table 1, Supplementary Fig. 2), a non-synonymous SNP in *FUT2* on chromosome 19p13.3, which has a minor allele frequency of 0.49 (Table 1). The association with plasma vitamin B₁₂ and rs602662 was independently replicated using Taqman allelic discrimination assays in 1,059 women from two nested case control studies drawn from the same cohort (Table 1, Supplementary Table 2) with $p_{\text{trend}} = 1.13 \times 10^{-06}$. The joint analysis of the CGEMS scan data and the replication dataset supports the association of rs602662 with plasma vitamin B₁₂ ($P_{\text{trend}} = 3.51 \times 10^{-15}$). Results were similar using untransformed plasma vitamin B₁₂ values. A similar association was observed using the non-parametric Kruskal-Wallis test (joint analysis Kruskal-Wallis $p = 1.48 \times 10^{-21}$). Plasma vitamin B₁₂ has an inverse correlation in this data set with plasma homocysteine, an integrated marker of the one-carbon metabolism pathway, (Spearman correlation coefficient -0.26 , $p < 0.0001$). *FUT2* rs602662 also has a modest association with plasma homocysteine, ($p_{\text{trend}} = 0.0085$ in the GWAS data and $p_{\text{trend}} = 0.0081$ in the replication data). The pattern of mean log-transformed plasma vitamin B₁₂ levels by rs602662 genotype suggest a dominant genetic effect, with lower B₁₂ levels among variant carriers (test comparing mean log-transformed vitamin B₁₂ levels between variant carriers and non-carriers $p = 1.35 \times 10^{-39}$).

The SNP rs602662 is in strong linkage disequilibrium ($D' = 1$, $r^2 = 0.76$) with the *FUT2* nonsense SNP *WI43X* (rs601338; Fig. 1). We imputed the nonsense SNP in the initial GWAS samples using the observed chromosome 19 genotyping data augmented by data from the densely-genotyped CEPH European HapMap samples and found that it was strongly associated with plasma vitamin B₁₂ levels ($p_{\text{trend}} = 4.11 \times 10^{-10}$; Fig. 1, Table 1). Since the nonsense SNP rs601338 could not be genotyped by Taqman because of a neighboring SNP (rs1800459) located one nucleotide upstream, we genotyped a proxy SNP, rs492602 (which is in perfect LD with rs601338 in HapMap data; Supplementary Table 1, Supplementary Fig. 3), in both the initial GWAS and the replication data set. The association between rs492602 and plasma vitamin B₁₂ was stronger than the association for rs602662 and plasma vitamin B₁₂ (Table 1: rs492602 joint $p_{\text{trend}} = 5.36 \times 10^{-17}$; joint dominant model $p = 8.26 \times 10^{-45}$). The rs492602 SNP explains 2.5% of the variance in log-transformed plasma vitamin B₁₂ levels using the log-additive genetic model and 3.5% of variance using the dominant genetic model.

Fucosylated carbohydrate structures are involved in a range of biological processes^{7,8} including tissue development, angiogenesis, fertilization, cell adhesion, inflammation, and tumor metastasis. The classic human secretor locus (*Se*) *FUT2* encodes $\alpha(1,2)$ fucosyltransferase, which regulates expression of the Lewis ABO(H) histo-blood group antigens on the surface of epithelial cells and in body fluids and determines the secretion status of the ABO antigens⁷. Secretor status of this polymorphic protein was used by Mohr to provide the first autosomal linkage in humans between secretor factor and the Lutheran blood group; subsequently secretor linkage was established with the APOE and myotonic dystrophy locus⁷. The family of α -1,2-fucosyltransferases catalyze the addition of fucose in α -1,2-linkage to the galactose of type 1 (Gal β 1,3GlcNAc-R) and type 2 Gal β 1,4GlcNAc- R) disaccharide to form H type 1 and H type 2 antigens⁷, respectively.

In the highly polymorphic *FUT2* gene⁷, three SNPs, rs602662, rs492602 and rs601338 (*WI43X*; nucleotide position 428), are in strong LD, we note that *WI43X* is a nonsense mutation⁷ and is plausibly the causal variant for the association with plasma B₁₂ levels. The *I43X* variant is characteristic for the nonsecretor allele in Europeans and has an allele frequency of 0.46 in populations of European ancestry⁷. In Europeans, Africans, and Iranians, the *FUT2* *WI43X* nonsense mutation is the primary nonsecretor allele, with a frequency of approximately 50 percent. In contrast, in Asian populations, the *FUT2* *I129F* (nucleotide position 385) missense mutation is the primary nonsecretor allele⁸. In non-Asian populations, nonsecretors are frequently homozygous for the *FUT2* *WI43X* polymorphism, resulting in an inactive *FUT2*. Individuals homozygous for the *FUT2* nonsecretor genotype appear to be resistant to infection with Norovirus⁹, suggesting that individuals homozygous for nonsecretor status may be unable to mediate host-microbe interactions⁹.

Absorption of B₁₂ requires the secretion of the glycoprotein intrinsic factor (IF) from the gastric cells, binding of IF to vitamin B₁₂ and a functional gastrointestinal absorption system². The H-antigen synthesized by *FUT2*, Lewis ABO antigens, and *FUT2* genotypes have all been reported to mediate *H. pylori* attachment to human gastric mucosa¹⁰. Atrophic gastritis is a consequence of *H. pylori* infection¹¹ and leads to reduced secretion of IF^{12,14}. The *FUT2* secretor status has been associated with both *H. pylori* infection and gastritis¹⁰; patients with vitamin B₁₂ malabsorption and low levels of serum vitamin B₁₂ have higher seroprevalence of *H. pylori* infection¹⁰. These data suggest a potential mechanism by which vitamin B₁₂ absorption^{12–15} may be reduced in carriers of the secretor genotype due to the sequelae of susceptibility to *H. pylori* infection compared to individuals with the nonsecretor genotype.

In summary, among generally well-nourished women, we found that common variation in the *FUT2* secretor gene is associated with plasma vitamin B₁₂ at high levels of statistical confidence. Although the participants included in these analyses are a selected subset of women who had plasma vitamin B₁₂ levels measured, numerous demographic and lifestyle factors are comparable between this sample and the overall NHS cohort. Insights gained from the study of plasma vitamin B₁₂ are likely to have implications for the study of complex diseases such as cognitive decline, cancer, and cardiovascular disease. Further study is required to investigate the biological basis of our reported association findings.

The commonly used abbreviations are

FUT2, Fucosyltransferase; GWAS, genome-wide association study; *H. pylori*, *Helicobacter pylori*; NHS, Nurses' Health Study; CI, confidence interval; SNPs, single nucleotide polymorphisms.

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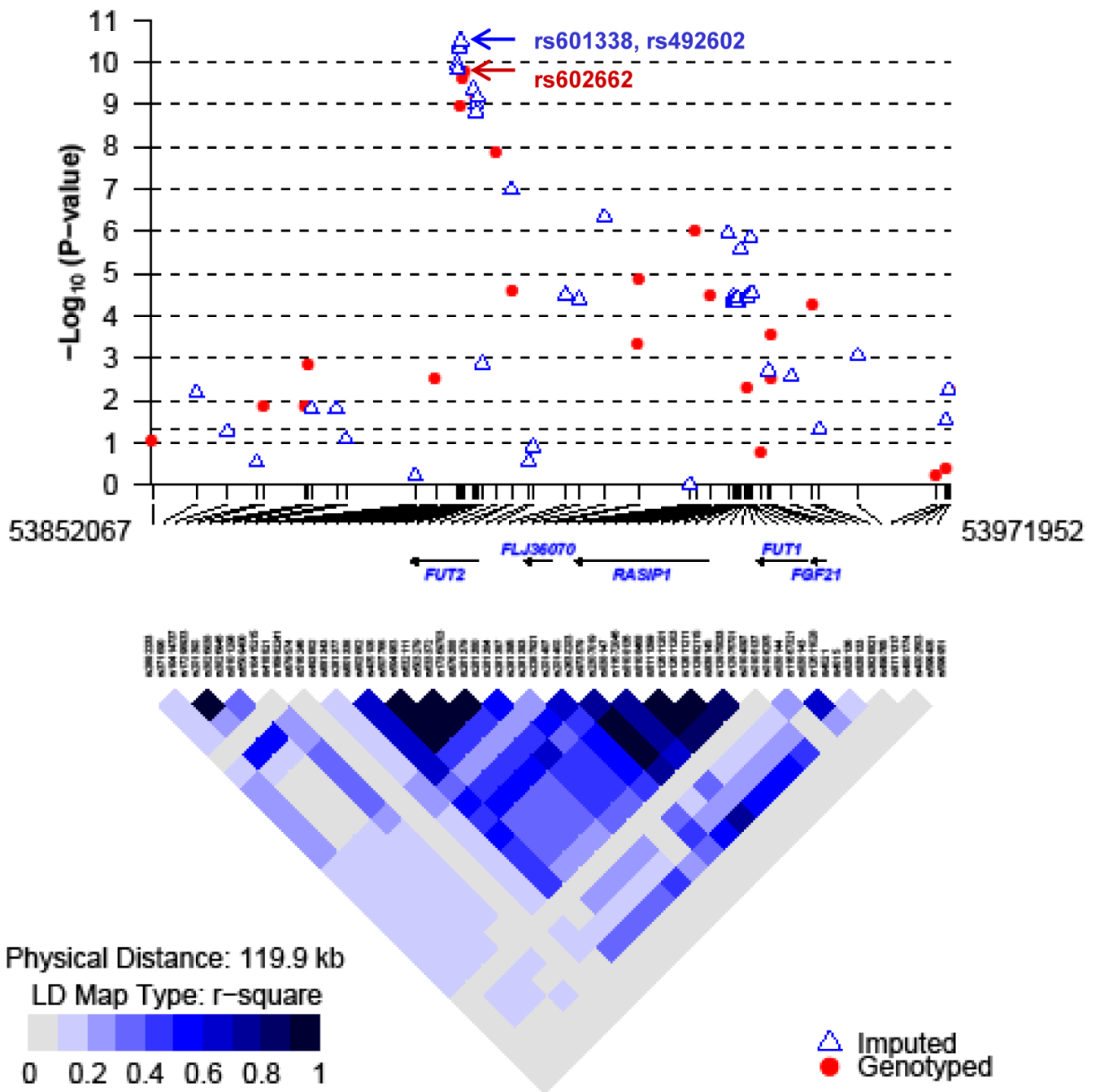


Figure 1. LD Structure of Chromosome 19

This panel shows the trend *P* values for association testing with plasma vitamin B₁₂ from 25 observed (●) and 44 imputed (△) SNPs from the GWA study displaying SNPs on chromosome 19p13.3. All known genes are shown (National Center for Biotechnology Information build 36.1)

Table 1
Association of plasma vitamin B₁₂ levels with *FUT2* genotypes in women participants from GWAS and replication studies

| SNP Association by Study | Age | N | Allele Freq | Geometric Mean (95% CI), pg/ml | | | Estimate (S.E.) ^c | P-value ^d |
|---|-----|-------|---------------------------|--------------------------------|-----------------------------|-------------------------------|------------------------------|--------------------------|
| <i>rs602662</i> GWAS | | | | Ser/Ser | Ser/Gly | Gly/Gly | | |
| NHS CGEMS | 59 | 1,658 | G (Gly)=0.49 | 489.82 (472.24–508.06) | 418.67 (407.74–429.90) | 417.05 (401.29–433.43) | –0.08 (0.01) | 6.54 × 10 ^{–10} |
| Replication | | | | | | | | |
| Combined NHS | 63 | 1,056 | | 483.68 (461.78–506.63) | 406.96 (393.69–420.67) | 409.60 (390.22–429.93) | –0.08 (0.02) | 1.13 × 10 ^{–06} |
| Colorectal (CR ^e) Adenoma and CR Cancer | | | | | | | | |
| Case-control Datasets | | | | | | | | |
| TOTAL (GWAS and Replication)^e | | | | 487.72 | 413.35 | 413.52 | –0.08 (0.01) | 3.52 × 10 ^{–15} |
| | | | | (473.80–502.06) | (404.83–422.06) | (401.14–426.27) | | |
| <i>rs601338^b</i> | | | ^a A (Trp)=0.51 | Nonsecretor (X/X) | Secretor (X/Trp) | Secretor (Trp/Trp) | | |
| GWAS | | | | | | | | |
| NHS CGEMS | 59 | 1,658 | | 496.60 (477.89–516.04) | 418.69 (407.76–429.92) | 418.32 (403.36–433.84) | –0.08 (0.01) | 4.11 × 10 ^{–10} |
| <i>rs492602</i> GWAS | | | ^a A=0.51 | GG/GG | AG/AG | AA/AA | | |
| NHS CGEMS | 59 | 1,637 | | 496.02 (477.32–515.45) | 419.95 (408.88–431.31) | 416.97 (402.14–432.35) | –0.08 (0.01) | 2.68 × 10 ^{–10} |
| Replication | | | | | | | | |
| Combined NHS CR Adenoma and CR Cancer | 63 | 1,059 | | 491.13 (469.03–514.26) | 407.67 (394.34–421.45) | 406.61 (389.46–424.53) | –0.10 (0.02) | 5.60 × 10 ^{–09} |
| Case-control Datasets | | | | | | | | |
| TOTAL (GWAS and Replication)^f | | | | 493.89 | 414.27 | 412.70 | –0.09 (0.01) | 5.36 × 10 ^{–17} |
| | | | | (479.42–508.81) | (405.69–423.04) | (401.33–424.40) | | |

^a Major allele according to HapMap CEU data

^b Imputed distribution for nonsense polymorphism, rs601338 (also known as W143X)

^c Estimates (regression coefficients) calculated from linear regression adjusted for age using log-transformed plasma vitamin B12 (same estimates obtained when adjusting for age, assay batch and total dietary methyl status)

^d P-values: GWAS and replication p-values are calculated from linear regression adjusted for age

^e colorectal is abbreviated as CR

^f Dominant model p-value for the joint analysis: rs602662 = 1.35×10^{-39} , rs492602 = 8.26×10^{-45}