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Genetic variation in folylpolyglutamate synthase and gamma-glutamyl hydrolase and plasma homocysteine levels in the Singapore Chinese Health Study

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Abstract

The enzymes folylpolyglutamate synthase (FPGS) and gamma-glutamyl hydrolase (GGH) are essential for determining intracellular folate availability for one-carbon metabolism (OCM) pathways. FPGS adds glutamyl groups to the folate molecule, thereby converting folate into the preferred substrate for several enzymes in OCM pathways. GGH removes glutamyl groups, allowing folate metabolites to leave the cell. The purpose of this study was to evaluate whether single nucleotide polymorphisms (SNPs) in the *FPGS* and *GGH* genes influence measured plasma homocysteine levels. Study participants were a sub-cohort (n = 482) from the Singapore Chinese Health Study. SNPs were selected using HapMap tagSNPs and SNPs previously reported in the scientific literature. Multiple linear regression was used to evaluate the association between individual SNPs and plasma homocysteine levels. Two *FPGS* (rs10106, rs1098774) and 9 *GGH* (rs719235, rs1031552, rs1800909, rs3758149, rs3780126, rs3824333, rs4617146, rs11545076, rs11545078) SNPs were included in the final analysis. Neither of the *FPGS* SNPs, but three *GGH* SNPs were associated with plasma homocysteine levels: rs11545076 (p=0.001), rs1800909 (p=0.02), and rs3758149 (p = 0.006). Only one (rs11545076) remained statistically significant after adjusting for multiple comparisons. This study suggests that *GGH* SNPs, rs11545076, rs1800909, and rs3758149, may have functional relevance and result in alterations in plasma homocysteine levels. Since this is one of the first studies to assess *FPGS* and *GGH* genetic

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variants in relation to plasma homocysteine, further research is needed to confirm these findings and characterize the functional effects of these variants.

Keywords

FPGS; GGH; Folate; Homocysteine; SNP

1 Introduction

Folates are important for one-carbon metabolism, where they act as one-carbon donors and acceptors. The widespread importance of one-carbon metabolism has made folate a vitamin of interest in the development of many chronic diseases, including cancer for its role in DNA synthesis, repair and methylation[1-4].

While many enzymes in the folate metabolism pathway have potential relevance for disease risk, it is also important to examine the enzymes that determine the availability of the substrates for the enzymes in these key pathways. Intracellular folate homeostasis is regulated by the addition or removal of glutamate residues to the γ -carboxylate group of folate (or folate derivatives) by folylpolyglutamate synthase (FPGS) or gamma-glutamyl hydrolase (GGH), respectively [5, 6]. The addition of glutamate groups increases cellular retention of folate. Additionally, many enzymes in the one-carbon metabolism pathway have a higher affinity for folate polyglutamates than for monoglutamates (main circulating form), thus the conversion between polyglutamate and monoglutamate forms can dictate the availability of substrates for key one-carbon metabolism enzymes (Figure 1) [5, 6].

To date, there is only one study that has examined the association between genetic variation in FPGS and GGH and biomarkers of one carbon metabolism. DeVos *et al* examined the relationship between one *FPGS* single nucleotide polymorphism (SNP) and two *GGH* SNPs in relation to plasma folate, plasma homocysteine and DNA uracil content. They found that the *GGH* rs11545076 homozygous variant GG genotype was associated with increased DNA uracil content, but was not associated with plasma folate or homocysteine levels. *FPGS* rs10106 and *GGH* rs3758149 were not significantly associated with any of the biomarkers [7].

The purpose of this study was to determine if SNPs in either *FPGS* or *GGH* are associated with variations in plasma homocysteine. The core hypotheses for this study were that variant alleles that cause higher FPGS activity would increase intracellular folate concentrations leading to lower homocysteine production and lower circulating homocysteine levels. Conversely, variant alleles that result in decreased FPGS expression would decrease intracellular folate concentrations resulting in increased plasma homocysteine. The inverse action of GGH led to the hypothesis that variant alleles that increase GGH activity would lead to higher plasma homocysteine levels, while those that decrease GGH activity will result in reduced homocysteine production.

2 Methods

2.1 Study Population

This study was conducted among participants in the Singapore Chinese Health Study, a population-based prospective cohort focused on investigating diet and cancer. The complete cohort contains 63,257 Chinese men and women who were 45-74 years of age at enrollment and were living in government housing estates. This particular analysis was done using a random sample of participants enrolled in the cohort after 1 year, from whom biospecimens

were collected ($n = 482$). This subsample was generally healthy; 24.9% had a history of hypertension at enrollment (Males = 27.6%, Females = 22.8%, $p = 0.23$), less than 10% had a history of coronary heart disease (Males = 5.1%, Females = 1.9%, $p = 0.05$), stroke (Males = 1.4%, Females = 1.1%, $p = 0.78$), diabetes (Males = 12.6%, Females = 7.1%, $p = 0.04$) and tuberculosis (Males = 3.7%, Females = 1.1%, $p = 0.06$). No participants in the subsample had a history of cancer. The Singapore Chinese Health Study was approved by the Institutional Review Boards of the National University of Singapore and the University of Minnesota.

2.2 Data Collection

The design of the Singapore Chinese Health Study has been previously described [8]. At the time of enrollment (between April 1, 1993 and December 31st, 1998), a face-to-face interview was conducted in the home of cohort participants by a trained interviewer using a structured questionnaire that requested information on demographics, lifetime use of tobacco (cigarettes and water-pipe), current physical activity, menstrual/reproductive history (women only), occupational exposure, medical history, and family history of cancer. Information on current diet, including alcohol consumption, was assessed via a 165-item food frequency questionnaire that has been validated against a series of 24-hour dietary recall interviews [8] and selected biomarker studies [9, 10] conducted on random subsets of cohort participants. The Singapore Food Composition Table, developed in conjunction with this cohort study, allows for the computation of intake levels of roughly 100 nutritive and non-nutritive compounds per study subject [8]. In April 1994, one year after the initiation of cohort subject recruitment, we began to collect blood and single-void urine specimens from a random 3% sample of study enrollees. Most blood samples were collected in the morning with no requirement for fasting. However, information on time of last meal was obtained. All blood specimens were processed and separated into their various components (plasma, serum, red cells, buffy coat) prior to storage at -80°C . The present study included 484 subjects with available plasma homocysteine, folate, vitamin B₆ and vitamin B₁₂ concentrations. The assays for plasma homocysteine and B vitamins were described previously [11]. Since kidney function can influence homocysteine levels, serum creatinine was measured using the Enzymatic Creatinine₂ (ECRE₂) method is based on the enzymatic reaction from Fossati *et al* [12].

2.3 Identification of SNPs

Few studies have been published regarding minor allele frequencies (MAF) of FPGS and GGH, particularly among the Singapore Chinese, thus, a multi-step SNP selection process was undertaken. Hap Map (<http://hapmap.ncbi.nlm.nih.gov/>) was used to identify haplotype tagging SNPs to include in the analysis. (Tagger-Pairwise, Hap Map Data Rel 27 Phase II + III, Feb09, on NCBI B36 assembly, dbSNP b126 for the CHB population). PubMed was used to identify additional SNPs in either the FPGS enzyme and/or GGH enzyme that had previously been reported in the literature to allow comparisons to previous research findings. Additionally, dbSNP (entrez SNP: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>) was used to obtain further information on SNPs, including location in the gene and to evaluate whether SNPs would be expected to alter enzyme expression and activity. SNPs that did not appear to show variation in the Han Chinese population according to population frequency data available on dbSNP and SNPPer (<http://snpper.chip.org/>) were excluded. However MAFs of many SNPs were unknown among the Singapore Chinese population, thus included in our initial genotyping.

A total of 11 FPGS (by rs number, any proxies are listed in parentheses): rs10106, rs2230270, rs10118903, rs10760502, rs10987742, rs11554717, rs17855900, rs34330923, rs34354111, rs35789560, rs41306702) and 17 GGH (rs15073, rs719235, rs1031552

(rs12677953), rs1800909, rs3758149, rs3824333, rs12681874, rs3780126, rs3780130, rs4617146, rs11545076, rs11545077, rs11545078, rs11786893, rs13270305, rs17898601) SNPs were included. General details about the SNPs included in the final analysis are presented in Supplementary Table 1.

2.4 Genotyping

DNA was extracted from buffy coats using a Qiagen QIAamp Kit (Qiagen Inc., Valencia, CA). Genotype determinations were performed in multiplex using the Sequenom MALDI-TOF mass spectrometry system (Sequenom Inc., San Diego, CA). Two of the FPGS SNPs were not genotyped; one due to lack of an adequate primer (rs3780130) and the other was a deletion mutation (rs71808601), which the Sequenom platform is not able to genotype. Upon further evaluation of the potential functional relevance of these SNPs, we did not expect these SNPs to alter enzymatic activity and they were subsequently excluded. Successful genotype results were obtained for 26 SNPs (11 FPGS, 15 GGH). We were unable to resolve >5% of genotype calls for three GGH SNPs (rs11545077, rs12681874, rs12677953). One of these three SNPs had a proxy that had been genotyped (rs12677953, $r^2 = 1$), and no data have been published that suggest the remaining two SNPs alter enzyme activity (rs11545077 and rs12681874). Several of the genotyped SNPs had a minor allele frequency (MAF) <0.02 in this population and were excluded from further analysis (FPGS: rs10118903, rs11554717, rs17855900, rs2230270, rs34330923, rs34354111, rs35789560, rs41306702, rs10760502 and GGH: rs11786893, rs15073). Quality control repeats for 10% of the genotype determinations showed 100% concordance.

2.5 Statistical Analysis

Participants who had missing values for plasma homocysteine and folate were excluded as were participants with extreme values for plasma folate, plasma homocysteine, serum creatinine and energy intake (>5000kcal or <500kcal). The distributions of plasma folate, homocysteine, vitamin B12 and Vitamin B6 and serum creatinine values were log-transformed to correct for markedly skewed distributions, therefore. Pearson correlations were used to assess the relationship between two continuous variables, tabular methods were used for comparing two categorical variables, and analysis of variance (ANOVA) was used for comparisons between a continuous and categorical variable. Multiple linear regression models were used to examine the relationship between genotype (explanatory variable) and plasma homocysteine concentrations. Variables were included in the full adjusted models if they were: 1) significantly associated with the outcome in the comparison analysis, 2) known to be potentially relevant (age, gender), or 3) previously found to be associated with plasma homocysteine in this cohort (MTHFR, tea consumption) [11]. Two previous studies have examined two other enzymes in the one carbon metabolism pathway in the same study population [11, 13]. Saw *et al* previously examined the association between *MTHFR* polymorphism C677T and homocysteine concentrations and observed a significant association between *MTHFR* genotype and homocysteine levels [11]. The other study by Trinh *et al* found that a thymidylate synthase (*TS*) polymorphism was not a significant determinant of homocysteine levels. For this reason, *MTHFR* C677T, but not *TS*, genotype was included in our multivariate regression models.

The final multivariate models were adjusted for age, gender, smoking status, plasma folate (log-transformed), plasma B₆ (log-transformed), plasma B₁₂ (log-transformed), serum creatinine, green tea intake and *MTHFR* C677T genotype. The critical p-values were adjusted for multiple comparisons using the Holm method [14]. This study had 80% power to detect a 0.363 nmol/L difference in homocysteine levels with a minor allele frequency of 0.20 ($\alpha = 0.05$, 2-sided, $n = 482$). All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc, Cary, NC).

3 Results

A total of 482 participants were included in the final analysis (214 men, 268 women). Average folate intake was higher in women ($105.5 \pm 34.0 \mu\text{g/d}$) than in men ($98.0 \pm 27.8 \mu\text{g/d}$), with naturally occurring food folates being the primary source rather than supplements or folic acid fortification. As a point of comparison, the United States Recommended Dietary Allowance (RDA) for folate is $400 \mu\text{g/d}$, [15] and Singapore's recommended daily allowance is $200 \mu\text{g/d}$. [16] Mean plasma folate was 13.5 nmol/L in men and 16.4 nmol/L in women, with an overall range from 4.2 to 46.5 nmol/L . Homocysteine levels ranged from 3.6 to 32.2 nmol/L with mean levels of $11.6 \pm 3.9 \text{ nmol/L}$ and $9.4 \pm 2.7 \text{ nmol/L}$ in men and women, respectively. At least once a week green tea only was consumed by 21.0% and 16.4%, black tea only by 26.2% and 12.7%, and both types by 11.2% and 11.9% of men and women, respectively. There were low rates of smoking and alcohol intake, but rates differed significantly between men and women. The prevalence of tobacco and alcohol use in women (less than 5% ever smoked or drank) was much lower than the prevalence in men (30.4% current smokers and 14% consumed alcohol at least weekly) (Table 1).

Dietary folate intake was positively associated with plasma folate ($r = 0.18$, $p < 0.001$) and negatively associated with plasma homocysteine ($r = -0.17$, $p < 0.001$). Plasma folate and homocysteine were negatively correlated with each other ($r = -0.47$, $p < 0.001$). Serum creatinine was positively correlated with homocysteine ($r = 0.51$, $p < 0.001$) and negative correlated with plasma folate ($r = -0.24$, $p < 0.001$). Homocysteine was positively correlated with age ($r = 0.29$, $p < 0.001$), inversely with B_6 intake ($r = -0.10$, $p = 0.02$), and not correlated with B_{12} intake ($r = -0.08$, $p = 0.07$). Smoking was negatively associated with plasma folate levels ($p < 0.001$) and positively associated with homocysteine levels ($p < 0.001$). Alcohol intake was not associated with plasma folate ($p = 0.47$) or homocysteine ($p = 0.40$). As described above, alcohol intake was low in this study population. While supplement intake was generally low, it was positively associated with plasma folate levels ($p = 0.01$), even with lack of information on specific types of dietary supplements that contain folate. Green tea intake was associated with homocysteine levels ($p = 0.03$), but the effect was not dose-dependent.

Genotype frequencies for all included SNPs were found to be in Hardy-Weinberg Equilibrium ($p > 0.001$) (Supplementary Table 2). MAFs for both the *FPGS* and most of the *GGH* SNPs were $>20\%$. Only two *GGH* SNPs had MAFs less than 20% (rs11545078 – 11% and rs719235 – 10%). MAFs for all SNPs are presented on Supplementary Table 2. Tables 2 and 3 summarize the findings of the genetic analyses. Neither of the two *FPGS* SNPs were associated with plasma homocysteine levels in the unadjusted or multivariate adjusted models regardless of adjustment for multiple comparisons (Table 2). The addition of *FPGS* genotype to the regression model accounted for approximately 2% of the variation in plasma homocysteine levels. Among the nine *GGH* SNPs assessed, three *GGH* SNPs (rs11545078, rs3758149, rs1800909) were significantly associated with plasma homocysteine levels. Adjustment for potential confounders strengthened the associations between these 3 SNPs and homocysteine levels (Table 3). For all three SNPs, the homozygous wild-type had the highest homocysteine levels with similar values for heterozygous and homozygous variants, although the differences in the mean plasma homocysteine levels between the three genotypes were quite small. The addition of the *GGH* SNPs to the models explained an additional 1.9 to 3.3% of the observed variation in plasma homocysteine levels. Tests for linkage disequilibrium suggest that these three SNPs are likely conveying similar information (r^2 : rs11545067*rs1800909 = 0.88, rs11545076*rs3758149 = 0.95, rs1800909*rs3758149 = 0.90). After adjusting for multiple comparisons, only rs11545076 remained statistically significant ($P = 0.001$).

4 Discussion

To our knowledge this is only the second study to assess SNPs in the folate pathway enzymes, *FPGS* and *GGH*, in relation to plasma homocysteine levels, and the first in a population without universal folate fortification and limited alcohol consumption. In this study, *FPGS* rs10106 and rs10987742 do not appear to influence plasma homocysteine levels. For rs10106, our findings are consistent with those of DeVos *et al.* *GGH* rs11545076, rs1800909, and rs3758149 did appear to slightly alter plasma homocysteine levels, but only rs11545076 remained statistically significant after adjusting for multiple comparisons. DeVos *et al* did not find rs11545076 to be associated with homocysteine concentrations, but they did find that the variant G allele was associated with increased DNA uracil content [7].

GGH rs11545076 and rs3758149 are both located upstream (–5') of the *GGH* gene. An *in vitro* study found that variant alleles for both SNPs increase promoter activity [17], suggesting the potential to influence enzyme levels, and ultimately, intracellular folate concentrations. The third SNP, rs1800909, is located in exon 1 and results in a missense mutation [18], however, bioinformatics prediction suggests that this is a benign amino acid change (Cys>Arg) [19]. Since these three SNPs do appear to be in linkage disequilibrium, it is possible that the observed variations in homocysteine levels could be due to genetic polymorphisms of one of the related SNPs alone or in combination, such as the haplotype. In this study, individuals carrying at least one variant allele had lower levels of plasma homocysteine than those homozygous for the wild-type, suggesting the variant allele results in decreased enzyme activity. Findings from the cell culture study by Chave *et al* indicate that variant alleles for rs11545076 and rs3758149 increase *GGH* expression. If the Chave *et al* findings are true, our observations of lower plasma homocysteine concentrations with the variant *GGH* alleles are not consistent with our hypothesis that higher *GGH* activity would lead to higher homocysteine levels. Additional research is needed to clarify this discrepancy.

There is some evidence from pharmacologic studies to indicate that alterations in *FPGS* and *GGH* function may alter cellular retention of folate. A study by Sadahiro *et al* suggests variation in *FPGS* and *GGH* can influence tissue folate levels after administration of 5-formyl THF (leucovorin) treatment for colorectal cancer [20]. Additionally, studies examining *FPGS* and *GGH* activity in relation to methotrexate (MTX), which is chemically similar to folate and taken into cells by the same mechanism as folate, suggest that decreased *FPGS* activity and increased *GGH* activity are associated with MTX resistance by limiting the amount of MTX retained in the cells [21, 22]. MTX retention in a cell uses the same polyglutamation mechanism as folate. MTX that is highly polyglutimated is retained in the cell, while short polyglutamate chains are associated with reduced cellular retention. Some studies have assessed the effects of SNPs in *FPGS* and *GGH* on MTX levels, efficacy and toxicity in rheumatoid (RA) and juvenile idiopathic arthritis patients [23–25]. Van der Straaten *et al* found no association with *FPGS* SNP rs10106 and MTX response in RA patients [25]. Consistent with our results, at three months this study suggests that the variant allele (CC) for rs1800909 may decrease enzyme activity, however, after 6 months no associations were observed. A second study examined associations between the SNP, rs1800909 and toxicity and efficacy of MTX in juvenile idiopathic arthritis patients [24]. This study found that liver dysfunction was associated with the heterozygous and variant homozygous (CC) rs1800909 genotypes, suggesting this polymorphism may reduce enzyme activity. This is consistent with our study results that found lower homocysteine levels (lower enzyme activity) among those heterozygous or homozygous (CC) variant for rs1800909. Dervieux *et al*, found that genotype for rs3758149 altered red blood cell levels of long chain polyglutamated MTX levels in RA patients. Participants homozygous for the variant TT genotype had lower levels of long chain polyglutamated MTX (increased enzyme activity) in red blood cells (OR: 4.8, 95% CI: 1.8 – 13.0) than homozygotes for the wild-

type or heterozygotes [23]. This is consistent with our results that suggest the wild-type (CC) for rs3758149 has lower activity than the homozygous variant (TT) genotypes with the highest homocysteine levels observed in those with the variant genotype. While these studies are assessing different questions, and assess glutamation of MTX instead of folate, the results of these studies are generally consistent with the results from this study.

There were some limitations in the current study. This study evaluated circulating plasma homocysteine levels as a surrogate for intracellular folate. Erythrocyte folate concentration data were not available, but would likely have been a better measure of intracellular folate levels, and more indicative of relative FPGS and GGH activity. Erythrocyte folate concentration would also allow for a more complete picture of long-term status than a single, cross-sectional measurement of plasma folate.

The observed differences in mean homocysteine among genotypes for the significant SNPs are small and would not be considered clinically meaningful. This could be related to the relatively similar homocysteine levels among participants, but it could also be due to the many other enzymes and transport proteins that could influence the levels of plasma homocysteine but were not evaluated in the present study. It is possible that alternative, non-folate dependent, homocysteine detoxification pathways may be more influential, or that additional genetic variants in other pathways interact with *FPGS* and *GGH* SNPs in relation to plasma homocysteine levels. Additionally, this study did not have sufficient numbers of participants to assess combinations of SNPs or to stratify the analyses by higher vs lower folate intake levels. Previous research has shown folate intake and genotype can interact, with genotype having different effects as high versus low folate levels. For example, studies of *MTHFR* C677T have shown that the variant T-allele is associated with increased risk of colon cancer when folate intake is low, but may be protective against colon cancer with sufficient folate intake [26].

This study also had several strengths. This was only the second study to assess the potential impact of genetic variation in *FPGS* and *GGH* on plasma homocysteine. This study differed from the previous study by DeVos *et al* in that our study population, Singapore Chinese, live in a country without mandatory folate fortification and tend not to drink alcohol or smoke cigarettes. The participants in the DeVos *et al* study were from the Puerto Rican community of Boston, and had higher prevalence of alcohol consumption and smoking [27]. Unlike DeVos *et al*, instead of evaluating many genes, we assessed multiple SNPs within only two genes, which allowed us to assess the potential influence of multiple SNPs for each gene.

More research is needed to understand the functional relevance of the *FPGS* and *GGH* SNPs. Future studies should aim to explore the impact of these genetic variants on long term folate status by using erythrocyte folate and multiple homocysteine measurements, since it is likely long term status that influences disease risk. It has been reported that expression of *GGH* is tissue specific [28], so when examining *GGH* and *FPGS* polymorphisms it may be important to take this into consideration.

4 Conclusions

This study suggests that *GGH* rs11545076, rs1800909, and rs3758149 may have functional relevance and result in alterations in plasma homocysteine levels in humans. Further research is needed to confirm these findings and characterize the functional effects of these variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- > We examine the impact of SNPs in FPGS and GGH genes on homocysteine levels.
- > Three GGH SNPs may influence homocysteine levels.
- > Functional changes have implications for cardiovascular disease and cancer research.
- > Functional changes have implications for methotrexate treatment response.

Figure 1. Folate Absorption, Cellular Uptake and Retention

Folate hydrolase 1 (FOLH1), proton-coupled folate transporter (PCFT), folate receptor 1 (FOLR1), reduced folate carrier 1 (RFC1), methionine synthase (MTR), Folylpolyglutamate synthase (FPGS), gamma glutamyl hydrolase (GGH).

Table 1

Characteristics of the study participants

	Men (N = 214)		Women (N = 268)		P
	Mean	SD	Mean	SD	
Age (yrs)	56.4	7.5	54.9	7.9	0.03
BMI (kg/m ²)	22.8	2.9	23.0	3.1	0.54
Caloric Intake (kcal/day)	1702.2	487.4	1402.3	466.6	<0.01
Folate Intake (µg/day) ^a	98.0	27.8	105.5	34.0	<0.01
Vitamin B12 Intake (µg/day) ^a	1.5	0.5	1.5	0.6	0.91
Vitamin B6 Intake (mg/day) ^a	0.7	0.2	0.7	0.2	0.69
Plasma Folate (nmol/L)	13.5	6.4	16.4	6.8	<0.01
Plasma Homocysteine (nmol/L)	11.6	3.6	9.4	2.7	<0.01
Plasma B ₁₂ (pmol/L)	328.7	130.4	380.4	199.3	<0.01
Plasma B ₆ (nmol/L)	36.1	28.1	44.2	31.6	<0.01
Serum Creatinine (µmol/L)	79.4	23.8	55.7	14.0	<0.01

	N	Percent	N	Percent	
Supplement Use					0.51
Yes	12	5.6	19	7.1	
No	202	94.4	249	92.9	
Smoker					<0.01
Never	95	44.4	256	95.5	
Past	54	25.2	2	0.8	
Current	65	30.4	10	3.7	
Alcohol Intake					<0.001
Never	146	68.2	249	92.9	
Monthly	26	12.2	10	3.7	
Weekly	30	14.0	6	2.3	
Daily	12	5.6	3	1.1	
Coffee Intake					0.02

	N	Percent	N	Percent
Never	35	16.4	47	17.5
Monthly/Weekly	34	15.9	31	11.6
1 cup/day	59	27.6	114	42.6
2-3 cups/day	78	36.4	70	26.1
4+ cups/day	8	3.7	6	2.2
Tea Intake				<0.01
None/Monthly	89	41.6	158	59.0
Black Tea	45	26.2	44	12.7
Green Tea	56	21.0	34	16.4
Both	24	11.2	32	11.9
Education				<0.01
No Formal Education	27	12.6	100	37.3
Primary School	101	47.2	99	36.9
Secondary School	59	27.6	57	21.3
A Level/Vocational	18	8.4	12	4.5
University	9	4.2	0	0

^aEnergy adjusted intake.

Table 2
FPGS: Adjusted and Unadjusted Mean Plasma Homocysteine by Genotype

SNP ^b	Unadjusted			Multivariate-Adjusted ^a				Holm α Level	R^2 ^d
	N	Mean ^c	95% CI	p-value	Mean ^c	95% CI	p-value		
rs10106				0.81			0.17	0.007	0.020
GG	229	9.8	8.9, 10.8		10.4	9.5, 11.4			
AG	193	10.0	9.6, 10.5		9.9	9.4, 10.5			
AA	40	9.8	9.5, 10.2		9.7	9.2, 10.2			
rs10987742				0.49			0.22	0.008	0.021
CC	257	9.8	9.4, 10.2		9.7	9.2, 10.2			
CT	184	10.1	9.7, 10.6		10.0	9.4, 10.6			
TT	36	9.7	8.8, 10.8		10.2	9.3, 11.2			

^aLinear regression-Adjusted for Age, Sex, Plasma Folate (log-transformed-continuous), Plasma B6 (log-transformed-continuous), Plasma B12 (log-transformed-continuous), Serum Creatinine (continuous), Smoking status (never, former, current smokers), Green Tea Intake (non-drinker, monthly, weekly, daily), and *MTHFR* C677T Genotype

^bOrdering for genotypes: 1. Wild-type homozygous, 2. Heterozygous, 3. Variant homozygous

^cGeometric Mean

^d R^2 is estimating the proportion of variance in plasma homocysteine levels explained by the SNP genotype in the adjusted model

Table 3
GGH: Adjusted and Unadjusted Mean Plasma Homocysteine by Genotype

SNP ^b	N	Unadjusted			Multivariate-Adjusted ^a				Holm α Level	R ^{2d}
		Mean ^c	95% CI	p-value	Mean ^c	95% CI	p-value			
rs1031552				0.08			0.37	0.02	0.020	
CC	202	9.6	9.2, 10.0		9.7	9.1, 10.2				
CT	219	10.1	9.7, 10.5		9.8	9.3, 10.4				
TT	54	10.4	9.6, 11.2		10.1	9.4, 10.9				
rs11545076				0.05			0.001	0.005	0.033	
TT	301	10.2	9.8, 10.5		10.1	9.6, 10.6				
GT	149	9.5	9.0, 10.0		9.3	8.8, 9.8				
GG	21	9.2	8.1, 10.5		9.6	8.6, 10.7				
rs1800909				0.03			0.02	0.005	0.022	
TT	304	10.2	9.9, 10.6		10.0	9.5, 10.6				
CT	142	9.4	9.0, 9.9		9.4	8.9, 10.0				
CC	21	9.5	8.4, 10.8		9.7	8.7, 10.8				
rs3758149				0.08			0.006	0.005	0.028	
CC	297	10.1	9.8, 10.5		10.0	9.5, 10.6				
CT	152	9.5	9.1, 10.0		9.3	8.8, 9.9				
TT	23	9.4	8.3, 10.7		9.4	8.5, 10.4				
rs3780126				0.35			0.21	0.01	0.022	
CC	190	10.0	9.6, 10.4		9.8	9.3, 10.4				
CT	228	9.7	9.3, 10.1		9.6	9.1, 10.2				
TT	57	10.3	9.5, 11.2		10.2	9.5, 11.0				
rs3824333				0.07			0.38	0.03	0.019	
AA	204	9.6	9.2, 10.0		9.7	9.1, 10.2				
AG	217	10.2	9.8, 10.6		9.9	9.3, 10.4				
GG	54	10.3	9.5, 11.2		10.1	9.2, 10.9				
rs4617146				0.24			0.28	0.006	0.020	
CC	172	10.1	9.7, 10.6		9.9	9.4, 10.5				
CT	223	9.9	9.6, 10.4		9.8	9.3, 10.4				

SNP ^b	Unadjusted				Multivariate-Adjusted ^a			
	N	Mean ^c	95% CI	p-value	Mean ^c	95% CI	p-value	Holm α Level
TT	81	9.4	8.8, 10.1		9.5	8.7, 10.1		
rs11545078				0.48			0.02	0.006
CC	379	10.0	9.7, 10.3		9.9	9.4, 10.4		
CT	88	9.6	9.0, 10.2		9.2	8.6, 9.8		
TT	7	10.0	8.0, 12.5		10.5	8.7, 12.7		
rs719235				0.72			0.41	0.01
GG	385	9.9	9.6, 10.2		9.7	9.2, 10.3		
GT	85	10.1	9.4, 10.7		10.0	9.4, 10.7		
TT	7	9.2	7.3, 11.5		9.1	7.6, 10.9		

^aLinear regression-Adjusted for Age, Sex, Plasma Folate (log-transformed-continuous), Plasma B6 (log-transformed-continuous), Plasma B12 (log-transformed-continuous), Serum Creatinine (continuous), Smoking status (never, former, current smokers), Green Tea Intake (non-drinker, monthly, weekly, daily), and MTHFR C677T Genotype

^bOrdering for genotypes: 1. Wild-type homozygous, 2. Heterozygous, 3. Variant homozygous

^cGeometric Mean

^dR² is estimating the proportion of variance in plasma homocysteine levels explained by the SNP genotype in the adjusted model