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Polymorphisms in folic acid metabolism genes do not associate with cancer cachexia in Japanese gastrointestinal patients

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ABSTRACT

We used clinical data from Iga General Hospital to examine the association between polymorphisms in *MTR* (*methionine synthase*) A2756G (rs1805087), *MTRR* (*methionine synthase reductase*) His595Tyr (rs10380), *MTHFR* (*methylenetetrahydrofolate reductase*) C677T (rs1801133), *MTHFR* A1298C (rs1801131) and *SHMT* (*serine hydroxymethyltransferase*) C1420T (rs1979277), which are genes involved in folate metabolism, and the risk of weight loss in patients with gastrointestinal cancers, with the aim of establishing personalized palliative care for each patient based on genetic information. The data from 59 patients (37 males and 22 females) with gastrointestinal cancers who visited the outpatient clinic for cancer chemotherapy and palliative care at Iga General Hospital from December 2011 to August 2015 were analyzed. There was no significant association between the single nucleotide polymorphisms (SNPs) in the folate metabolizing genes examined and weight loss defined as weight loss of more than 5 percent or more than 10 percent during the first 6 months after initiation of chemotherapy. We did not detect any significant association between any of the SNPs examined and overall survival of patients. The present study indicated that these SNPs have relatively limited or no roles in the genesis of cachexia in patients with gastrointestinal cancers; however, further investigations into the roles of these folate metabolizing genes in the context of cancer palliative care, from clinical, biological and epidemiological viewpoints are warranted.

Keywords: gastrointestinal cancer, single nucleotide polymorphism, folic acid metabolism, cancer cachexia

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INTRODUCTION

Cancer is one of the major causes of death worldwide, and it is the most common cause of death in Japan. Inflammation, anorexia and resulting weight loss and muscle wasting, (termed sarcopenia), because of decreased nutrient intake are frequently observed symptoms in cancer patients. Such symptoms are known as "cachexia", which is a characteristic disorder of cancer patients especially in advanced clinical stages such as UICC (Union for International Cancer Control) stage 4. Cachexia reduces quality of life and usually patients require palliative clinical support. Tumor existence and progression play key roles in causing cachexia and some genetic polymorphisms are possible causes of cachexia.

Folate deficiency may increase the incidence of cancers.[1)] The destabilization of DNA may lead to chromosome aberrations and potentially malignant transformation. Reduced methylation of cytosine in DNA because of folate deficiency might also result in the expression of pro-oncogenes and potential malignant transformation.[1)] Also, high dietary folate intake is associated with a reduced risk of mortality from stroke, coronary heart disease, and heart failure in the Japanese population.[2)] One recent Japan-based study demonstrated that folate intake and folate-related genetic polymorphisms may affect the efficacy of fluorouracil (FU)-based chemotherapy in advanced gastric cancer.[3)]

Iga General Hospital in central Japan provides palliative care to patients with gastrointestinal cancers, and has accumulated clinical data, including body weight, muscle weight and weight of total body water/total body fat of hospital patients. We analyzed the body composition of the cancer patients who visited the outpatient clinic once a month. Here, we used clinical data from Iga General Hospital to examine the association between polymorphisms in *MTR* (*methionine synthase*) A2756G (rs1805087), *MTRR* (*methionine synthase reductase*) His595Tyr (rs10380), *MTHFR* (*methylenetetrahydrofolate reductase*) C677T (rs1801133), *MTHFR* A1298C (rs1801131) and *SHMT* (*serine hydroxymethyltransferase*) C1420T (rs1979277) genes, which are involved in folate metabolism, and the risk of weight loss in patients with gastrointestinal cancers with the aim of establishing personalized palliative care for each patient based on genetic information.

MATERIALS AND METHODS

Data from 59 patients with gastrointestinal cancers (37 males and 22 females) who visited the outpatient clinic for cancer chemotherapy and palliative care at Iga General Hospital from December 2011 to August 2015 were analyzed. Fifty-three patients underwent surgery and six did not. All patients underwent palliative chemotherapy and their body composition (total body weight, skeletal muscle weight and water weight) was measured, mainly to monitor cachexia. All of the patients gave written informed consent and provided clinical data and blood for analysis and DNA testing. Patients' weight loss in the 6 months after the initiation of chemotherapy was categorized as weight loss of more than 5 percent (WL5) or of more than 10 percent (WL10). Follow up of patients' clinical information/conditions were conducted by checking their electronic and/or paper medical records.

DNA was extracted from the patients' buffy coat, using the Qiagen Bio Robot EZ1 (Qiagen, Hilden, Germany). Genotyping of *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131), *MTR* A2756G (rs1805087), *MTRR* His595Tyr (rs10380) and *SHMT* C1420T (rs1979277) was performed by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).[4)] The primers and the thermocycler conditions used for each SNP are described in Table 1. Representative genotyping gels are shown in Fig. 1.

Table 1 PCR primers and conditions used.

SNP	Primer		PCR				Amplicon sizes (bp)	
	Sequence	conc. (μM)	Temp (°C)	min		cycles	Genotype	Size (bp)
MTHFR C677T (rs1801133)	F1: GAG AAG GTG TCT GCG GGA GT	0.5	95	10		1	*C/C*	128 & 183
	R1: CAT GTC GGT GCA TGC CTT	0.5	95	1			*C/T*	93, 128 & 183
	F2: AGC CTC TCC TGA CTG TCA TCC	0.5	60	1	}	30	*T/T*	93 & 183
	R2: TGC GTG ATG ATG AAA TCG G	0.5	72	1				
			72	5		1		
MTHFR A1298C (rs1801131)	F1: GGA GGA GCT GAC CAG TGA AGA	0.5	95	10		1	*A/A*	206 & 284
	R1: CCA TGT CCA CAG CAT GGA G	0.5	95	1			*A/C*	124, 206 & 284
	F2: CTT TGG GGA GCT GAA GGA CTA CTA	0.5	60	1	}	30	*C/C*	124 & 284
	R2: AAG AAC GAA GAC TTC AAA GAC ACT TG	0.5	72	1				
			72	5		1		
MTR A2756G (rs1805087)	F1: ATG GAA GAA TAT GAA GAT ATT AGA CAG GG	0.5	95	10		1	*G/G*	162 & 355
	R1: GAT CCA AAG CCT TTT ACA CTC CTC	0.5	95	1			*G/A*	162, 251 & 355
	F2: ACG CCA GGC AGG AAT TAG C	0.5	65	1	}	30	*A/A*	251 & 355
	R2: CCA CTT ACC TTG AGA GAC TCA TAA TGG T	0.5	72	1				
			72	5		1		
MTRR His595Tyr (rs10380)	F1: CCA TTA TAT ATT ATA TTT CAG AAA AGA GCT CAG AC	0.5	95	10		1	*C/C*	300 & 498
	R1: ACA TAT CTA GAT TAT TGG CTT CTC GGT C	0.5	95	1			*C/T*	199, 300 & 498
	F2: ATG TGG TTG TTT TTT GGC TGC	0.5	63	1	}	30	*T/T*	199 & 498
	R2: GAG TTA AGA TCC CAT GCT TAA GGA AAT A	0.5	72	1				
			72	5		1		
SHMT C1420T (rs1979277)	F1: GAG GTT GAG AGC TTC GCC TCT T	0.5	95	10		1	*C/C*	215 & 515
	R1: GCT GCC CAG GAC ATC TGT C	0.5	95	1			*C/T*	215, 337 & 515
	F2: TGA TTT GTG AAG AAA ACA TGA AAA AAG TC	0.5	65	1	}	30	*T/T*	337 & 515
	R2: GCC AGG CAG AGG GAA GAG	0.5	72	1				
			72	5		1		

PCR, polymerase chain reaction; bp, base pairs.
The PCR conducted was polymerase chain reaction with confronting two-pair primers (PCR-CTPP) [3]. The polymorphic bases are underlined.

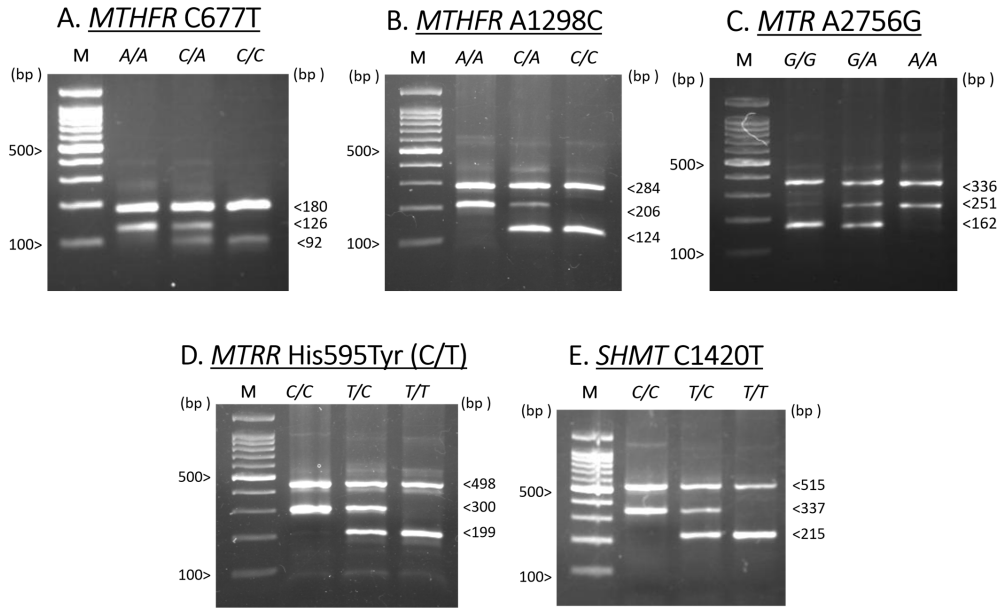

Fig. 1 Representative genotyping gels

A. *MTHFR* A1298C (rs1801131): Lane M, 100-bp marker; left lane, *C/C* genotype (128-bp and 183-bp bands); middle lane, *C/T* genotype (93-bp, 128-bp and 183-bp bands); right lane, *T/T* genotype (93-bp and 183-bp bands).

B. *MTHFR* A1298C (rs1801133): Lane M, 100-bp marker; left lane, *A/A* genotype (206-bp and 284-bp bands); middle lane, *C/A* genotype (124-bp, 206-bp and 284-bp bands); right lane, *C/C* genotype (124-bp and 284-bp bands).

C. *MTR* A2756G (rs1805087): Lane M, 100-bp marker; left lane, *G/G* genotype (162-bp and 355-bp bands); middle lane, *G/A* genotype (162-bp, 251-bp and 355-bp bands); right lane, *A/A* genotype (251-bp and 355-bp bands).

D. *MTRR* His595Tyr (rs10380): Lane M, 100-bp marker; left lane, *C/C* genotype (300-bp and 498-bp bands); middle lane, *T/C* genotype (199-bp, 300-bp and 498-bp bands); right lane, *T/T* genotype (199-bp and 498-bp bands).

E. *SHMT* C1420T (rs1979277): Lane M, 100-bp marker; left lane, *C/C* genotype (115-bp and 144-bp bands); middle lane, *T/C* genotype (68-bp, 115-bp and 144-bp bands); right lane, *T/T* genotype (68-bp and 144-bp bands).

The statistical software, STATA ver.13 (STATA Corp, TX), was used for analysis. To evaluate the effects of genetic polymorphisms on patient survival, the Kaplan-Meier Curve, the logrank test, the Wilcoxon test and the Cox proportional hazard model were used. We analyzed the effect of genotype on patient body weight using the logistic regression model. This study was approved by the Institutional Review Board of Nagoya University Graduate School of Medicine (approval no.2013-0220-08).

RESULTS

Table 2 shows patient characteristics (age, sex, cancer type, UICC stage, genotype frequency). The major cancer types were colorectal (n = 40; 67.8%) and stomach (n = 11; 18.6%), and the majority of patients were in advanced clinical stages, at UICC stage III or IV (n = 44; 74.6%).

Table 3 shows the risk of weight loss (WL5 and WL10) by genotype with the crude ORs (odds ratios) and the aORs (adjusted odds ratios) for each genotype. There was no significant association between the SNPs examined and weight loss (WL5 or WL10). Table 4 shows logrank P values, crude HRs (hazard ratios) and aHRs (adjusted hazard ratios) by genotype. No significant association between any of the folate metabolizing gene SNPs and overall patient survival was observed (Table 4 & Fig. 2).

Table 2 Patient characteristics

Variables		Male (n = 37)	Female (n = 22)
Age [y (sd)]			
		67.9 (14.4)	68.4 (7.6)
Cancer Type [n (%)]			
	Esophageal	2 (5.4)	0 (0.0)
	Stomach	7 (18.9)	4 (18.2)
	Colorectal	26 (70.3)	14 (63.6)
	Pancreatic	1 (2.7)	4 (18.2)
	Billiary	1 (2.7)	0 (0.0)
UICC Stage [n (%)]			
	I	4 (10.8)	1 (4.5)
	II	6 (16.2)	4 (18.2)
	III	10 (27.0)	5 (22.7)
	IV	17 (46.0)	12 (54.5)
Genotype Frequency			
	MTHFR C677T (rs1801133)		
	C/C	13 (35.1)	7 (31.8)
	C/T	17 (46.0)	9 (40.9)
	T/T	7 (18.9)	6 (27.3)
	MTHFR A1298C (rs1801131)		
	A/A	28 (75.7)	14 (63.6)
	A/C	9 (24.3)	7 (31.8)
	C/C	0 (0.0)	1 (4.6)
	MTR A2756G (rs1805087)		
	A/A	24 (64.9)	10 (45.5)
	A/G	13 (35.1)	12 (54.5)
	G/G	0 (0.0)	0 (0.0)
	MTRR His595Tyr (rs10380)		
	C/C	31 (83.8)	18 (81.8)

	C/T	6 (16.2)	3 (13.6)
	T/T	0 (0.0)	1 (4.6)
SHMT C1420T (rs1979277)			
	A/A	26 (70.3)	16 (72.7)
	A/C	10 (27.0)	6 (27.3)
	C/C	1 (2.7)	0 (0.0)

Table 3 Risk of weight loss by genotype

	WL	WL (–)	crude OR	aOR-1	aOR-2
(WL5)					
MTHFR C677T (rs1801133)					
C/C	10	8	1	1	1
C/T	15	8	1.50 (0.42–5.32)	1.47 (0.41–5.26)	1.73 (0.44–6.80)
T/T	7	3	1.87 (0.36–9.63)	1.83 (0.35–9.54)	2.50 (0.43–14.63)
C/T + T/T	22	11	1.60 (0.49–3.17)	1.57 (0.48–5.13)	1.93 (0.54–6.92)
MTHFR A1298C (rs1801131)					
A/A	21	15	1	1	1
A/C + C/C	10	4	1.70 (0.45–6.46)	1.62 (0.42–6.23)	0.79 (0.16–3.83)
MTR A2756G (rs1805087)					
A/A	19	11	1	1	1
A/G + G/G	13	8	0.94 (0.30–2.98)	0.87 (0.27–2.84)	1.37 (0.37–5.12)
MTRR His595Tyr (rs10380)					
C/C	28	15	1	1	1
C/T + T/T	4	4	0.20 (0.02–1.76)	0.53 (0.12–2.46)	0.40 (0.08–2.02)
SHMT C1420T (rs1979277)					
A/A	22	14	1	1	1
A/C + C/C	10	5	1.27 (0.36–4.51)	1.30 (0.37–4.66)	1.45 (0.38–5.54)
(WL10)					
MTHFR C677T (rs1801133)					
C/C	7	11	1	1	1
C/T	8	15	0.84 (0.23–3.01)	0.84 (0.23–3.07)	0.94 (0.24–3.66)
T/T	4	6	1.05 (0.22–5.09)	1.01 (0.21–4.95)	1.26 (0.23–6.83)
C/T + T/T	12	21	0.90 (0.27–2.93)	0.89 (0.27–2.94)	1.03 (0.29–3.62)
MTHFR A1298C (rs1801131)					
A/A	13	23	1	1	1
A/C + C/C	6	8	1.38 (0.39–4.86)	1.32 (0.37–4.72)	0.68 (0.16–2.85)
MTR A2756G (rs1805087)					
A/A	12	18	1	1	1

A/G + G/G	7	14	0.75 (0.23–2.40)	0.71 (0.21–2.36)	1.04 (0.28–3.80)
MTRR His595Tyr (rs10380)					
C/C	18	25	1	1	1
C/T + T/T	1	7	0.19 (0.02–1.75)	0.20 (0.02–1.76)	0.16 (0.02–1.46)
SHMT C1420T (rs1979277)					
A/A	13	23	1	1	1
A/C + C/C	6	9	1.18 (0.34–4.06)	1.20 (0.35–4.18)	1.31 (0.35–4.88)

WL: weight loss. WL5 (10) indicates the number of subjects with weight loss of more than 5% (or 10%); WL (–) indicates patients with no weight loss.
OR: odds ratio (95% confidence interval in parentheses); aOR, adjusted odds ratio (adjusted for age and sex); aOR-2, adjusted odds ratio (adjusted for age, sex and clinical stage [stage 4]).
**Per-genotype* analyses for the *MTHFR* A1298C (rs1801133), *MTR* A2756G (rs1805087), *MTRR* His595Tyr (rs10380) and *SHMT* C1420T (rs1979277) SNPs were omitted because of the small number of patients in the strata of those who are homozygous for the minor allele.

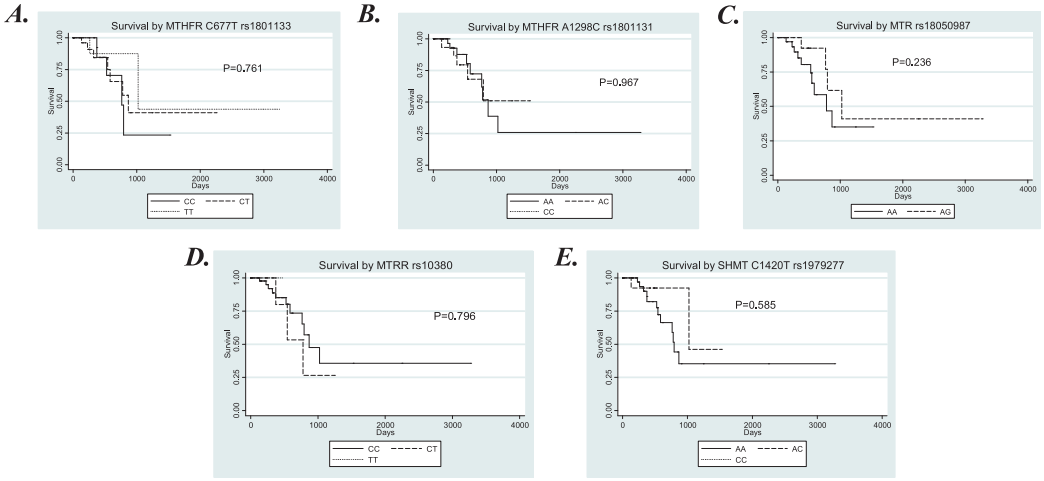

Fig. 2 Association of survival with genotype
A: Association of survival with *MTHFR* C677T
B: Association of survival with *MTHFR* A1298C
C: Association of survival with *MTR* A2756G
D: Association of survival with *MTRR* His595Tyr
E: Association of survival with *SHMT* C1420T
*P values indicate logrank P values.

Table 4 Patient survival by genotype

Comparison groups	Logrank *P*	Model	Crude HR	aHR-1	aHR-2	aHR-3
MTHFR C677T (rs1801133)						
all 3 genotypes	0.761	*additive*	0.75 (0.35–1.62)	0.74 (0.35–1.59)	0.97 (0.48–1.97)	0.58 (0.20–1.61)
vt. hetero+vt. homo vs. wt. homo	0.621	*dominant*	0.75 (0.24–2.30)	0.79 (0.26–2.45)	1.75 (0.49–6.18)	0.90 (0.13–6.01)
vt. homo vs. others	0.494	*recessive*	0.59 (0.13–2.68)	0.47 (0.09–2.40)	0.40 (0.08–2.03)	0.28 (0.04–1.57)
MTHFR A1298C (rs1801131)						
all 3 genotypes	0.967	*additive*	0.90 (0.30–2.67)	0.97 (0.32–2.91)	0.66 (0.21–2.05)	1.01 (0.277–3.74)
vt. hetero+vt. homo vs. wt. homo	0.869	*dominant*	0.91 (0.30–2.73)	0.99 (0.32–2.99)	0.67 (0.21–2.09)	1.09 (0.27–4.37)
MTR A2756G (rs1805087)						
all 3 genotypes	0.236	*additive*	0.49 (0.15–1.61)	0.43 (0.13–1.44)	0.41 (0.11–1.42)	0.39 (0.07–2.23)
vt. hetero+vt. homo vs. wt. homo	0.236	*dominant*	0.49 (0.15–1.61)	0.43 (0.13–1.44)	0.41 (0.11–1.42)	0.39 (0.07–2.23)
MTRR His595Tyr (rs10380)						
all 3 genotypes	0.796	*additive*	1.18 (0.36–3.84)	1.56 (0.47–5.13)	2.52 (0.62–10.2)	5.90 (0.78–44.3)
vt. hetero+vt. homo vs. wt. homo	0.67	*dominant*	1.31 (0.36–4.74)	2.02 (0.50–8.12)	3.10 (0.66–14.5)	6.65 (0.80–55.1)
SHMT C1420T (rs1979277)						
all 3 genotypes	0.743	*additive*	0.55 (0.12–2.52)	0.48 (0.10–2.39)	0.16 (0.02–1.12)	0.23 (0.006–8.68)
vt. hetero+vt. homo vs. wt. homo	0.441	*dominant*	0.55 (0.12–2.52)	0.48 (0.10–2.39)	0.16 (0.02–1.12)	0.23 (0.006–8.68)

HR, hazard ratio; aHR, adjusted hazard ratio (aHR-1, adjusted for sex and age; aHR-2, adjusted for age, sex and clinical stage [stage 4]; aHR-3, adjusted for age, sex, clinical stage and cancer type [coded as indicator variables: variables assigned for each cancer type and coded as 1 for presence and 0 for absence]).
*Analysis with the recessive model for *MTHFR* A1298C (rs1801133), *MTR* A2756G (rs1805087), *MTRR* His595Tyr (rs10380) and *SHMT* C1420T (rs1979277) were omitted because of the small number of patients in the strata of those who are homozygous for the minor allele.

DISCUSSION

Many causes of cancer have been reported or are under debate and, as described before, folate deficiency is associated with cancer incidence. *MTHFR*, *MTR*, *MTRR*, and *SHMT* are involved in folate metabolism, meaning that polymorphisms in these genes are potential risk modulators of cancer. In addition to roles in the development of various cancers, such as breast and cervical cancer, folate metabolism also plays roles in other diseases, such as cardiovascular and coronary artery disease.[1)]

The *MTR* gene encodes the enzyme methionine synthase.[5)] Reduced activity of *MTR* because of the *MTR* A2756G polymorphism might lead to compromised conversion of homocysteine to methionine. Subsequently, homocysteine levels in the bloodstream may rise and methionine levels may fall.[5)] The *MTR* A2756G polymorphism is also associated with risk of retinoblastoma in Iranian patients.[6)]

The *MTRR* gene encodes an enzyme called methionine synthase reductase,[7)] which reactivates methionine synthase. *MTRR* His595Tyr may, therefore, affect the production of methionine.[7)] It is

also reported that *MTRR* His595Tyr is associated with the maternal risk for Down syndrome.[8] Taken together, *MTR* A2756G and *MTRR* His595Tyr may cooperate to affect methionine metabolism and disease risk.

The *MTHFR* gene encodes the enzyme methylenetetrahydrofolate reductase, which is required for the multistep process that converts the amino acid homocysteine into another amino acid, methionine.[9] *MTHFR* polymorphisms are associated with the risk of vascular diseases, psoriasis, infertility, neurological diseases and various cancers.[1] Folate levels in combination with the *MTHFR* C677T genotype are associated with the expression of miR-21, a small non-coding RNA that regulates gene expression and that is often packaged within secreted microvesicles.[10,11] Tumor-derived microvesicles induce apoptosis of skeletal muscle cells, resulting in the loss of skeletal muscle mass, which is a characteristic symptom of cancer cachexia.[10] The T allele of the *MTHFR* C677T (rs1801133) polymorphism reportedly corresponds to higher serum miR-21 levels,[11] which could theoretically modulate the risk of cancer cachexia in humans. In the present study, however, there was no statistically significant association between *MTHFR* C677T and weight loss, presumably because of the small number of subjects examined. Further investigations with larger sample sizes are required. *SHMT* catalyzes the reversible reaction of serine and tetrahydrofolate (THF) to glycine and 5, 10-methylene THF.[12] The *SHMT* C1420T polymorphism may cause a shift in the distribution of the different folate derivatives.[12] There is a possible association between serum folate levels and body weight reduction in cancer patients. Moreover, genetic variations in folate metabolism have important roles in the prognosis of cancer patients;[13-15] however, associations of genetic variations in folate metabolizing genes with risk of weight loss in cancer patients remain to be clarified. The extent of anorexia or appetite loss may also differ according to the genotypes of folate metabolizing genes, presumably because of differential severities of chemotherapy side effects,[3,14,15] which may also influence the risk of weight loss.

The allele frequencies of the SNPs were not significantly different from reported frequencies, except for *SHMT* rs1979277 (P = 0.122 and χ^2 = 2.397 for *MTHFR* rs1801133 [*C* allele frequency = 0.640, *T* = 0.360; n = 172 from the HapMap JPT data of dbSNP];[16] *P* = 0.569 and χ^2 = 0.325 for *MTHFR* rs1801131 [*A* = 0.822, *C* = 0.178; n = 90]; *P* = 0.972 and χ^2 = 0.001 for *MTR* rs1805087 [*A* = 0.787, *C* = 0.213]; *P* = 0.755 and χ^2 = 0.098 for *MTRR* His595Tyr [*C* = 0.890, *C* = 0.110]; and *P* = 0.002 and χ^2 = 10.049 for *SHMT* rs1979277 [*T* = 0.045, *C* = 0.955], χ^2 test). The significant difference observed in *SHMT* rs1979277 SNP may result from the small sample size in the present data set, or the existence of etiological mechanisms between this SNP and gastrointestinal cancers.

In this study, 59 patients suffering from gastrointestinal cancers of various organs were analyzed, and there was no statistical significance observed, suggesting that the roles of these SNPs in folate metabolizing pathways are relatively limited in the genesis of cachexia in patients with gastrointestinal cancers. The statistical power considerations for the present data set can be described as follows. The statistical power for 59 subjects is more than 50% for an OR of 4 or 0.25 with a two-sided α-error of 0.05, when a genotype frequency among the controls was between 40% and 60% with the allocation ratio of 1.5:1 – 0.67:1 by case/control status. With regard to the survival analysis, the statistical power was more than 90% for an HR of 3 or 0.33 with a two-sided α-error of 0.05, when a genotype frequency was between 33% and 67%, while it was more than 55% for an HR of 2 or 0.5 under the same conditions. However, further study with larger sample sizes may lead to replication of the previously reported association of *MTHFR* SNPs and gastric cancer prognosis, or may find a yet unknown association of genetic variations in folate metabolizing genes with clinical outcomes of patients with gastrointestinal cancers. Further investigations of the roles of these folate metabolizing genes in the context of

cancer palliative care, from clinical, biological and epidemiological viewpoints are warranted.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose with regard to this manuscript.

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