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Clinical Impact of the *AKT1* rs1130233 SNP in Japanese Gastrointestinal Cancer Patients with Palliative Care

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Abstract

Objective: Cancer patients often suffer from chronic inflammation, anorexia and the resultant decrease of nutrient intake, followed by weight loss and muscle wasting called “sarcopenia”. Such conditions are known as “cachexia”. In this study, we examined the associations between genetic polymorphisms of *AKT1* rs1130233, *ICAM1* rs281432, *SELP* rs6128 and *TNSRSF1A* rs4149570, which are reportedly associated with cachexia in Caucasians, together with *LIF* rs929271, in Japanese gastrointestinal cancer patients with palliative care.

Methods: The study subjects were 59 patients (37 males and 22 females) with gastrointestinal cancers who visited the outpatient clinic at Iga General Hospital from December 2011 till August 2015. Genotypings for *AKT1* rs1130233, *ICAM1* rs281432, *SELP* rs6128, *TNSRSF1A* rs4149570 and *LIF* rs929271 were conducted with polymerase chain reaction with confronting two-pair primers (PCR-CTPP) or the Taqman SNP Genotyping assay. Associations of these SNPs with patients' prognosis as well as weight loss defined as weight loss more than 5 percent during 6 months after the initiation of chemotherapy were evaluated.

Results: A significant increase in the risk of 5% weight loss was observed in those with A/G genotype *AKT1* rs1130233 polymorphism (*AKT1* A/G vs. G/G, adjusted odds ratio [aOR]=7.11; 95%CI: 1.41-35.7), or in those with at least one A allele of *AKT1* rs1130233 (*AKT1* A/G+A/A vs. G/G, aOR=4.57; 95% CI: 1.14-18.3) when adjusted for age, sex and UICC clinical stage 4. There was no statistically significant correlation of the polymorphisms examined with patients' survival.

Conclusion: The present study revealed that *AKT1* rs1130233 A allele may play a key role in the development of cancer cachexia. Given the involvement of *AKT1* in the development of cancer as well as in apoptosis, it would be worth studying the roles of this molecule in human cancers further from clinical, epidemiological and biological viewpoints in the near future.

Keywords: Gastrointestinal cancer; Cancer cachexia; Genetic polymorphisms; AKT serine/threonine kinase 1; Weight loss; Apoptosis; Cancer palliative care

Introduction

Cancer is one of the major causes of morbidity and mortality worldwide, and one of the leading causes of deaths in Japan. Gastrointestinal cancers explain the largest part of cancer incidence and mortality in Japan [1], making the palliative care to the patients with these life-threatening diseases increasingly important in recent years. Cancer patients often suffer from chronic inflammation, anorexia and the resultant decrease of nutrient intake, followed by weight loss and muscle wasting called “sarcopenia”. Such conditions are known as “cachexia”, which worsens patients' quality of life, pressing the need for palliative medical care. It is usually induced by the tumor existence and progression, and some genetic polymorphisms are reported as possible causes of cachexia [2,3].

Iga General Hospital in the central of Japan is one of the key hospitals providing palliative care to the corresponding area. There are large amount of patients' clinical data such as weight, muscle weight of the patients collected and accumulated in the hospital. *AKT1* (AKT serine/threonine kinase 1) and *SELP* (selectin P) polymorphisms are involved in cancer cachexia in pancreatic patients in Caucasians [4]. Polymorphisms of *ICAM1* (intercellular adhesion molecule 1)

can be a potentially useful biomarker for identifying individuals with higher risk of gastric cancer, predicting disease progression, and guiding individualized treatment [5]. One *TNSRSF1A* (tumor necrosis factor receptor superfamily 1A) polymorphism is reportedly an important risk marker for T-cell lymphoma via the constitutively elevated TNF- α (tumor necrosis alpha) expression [6]. Recently, *LIF* (leukemia inhibitory factor) was demonstrated to induce muscle atrophy in the mouse model of colon cancer [7], and the SNP (single nucleotide polymorphism) of *LIF* rs929271 T/G has been shown to be a susceptibility biomarker capable of predicting implantation efficiency and pregnancy outcomes [8].

In the present study, we examined the associations between genetic

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polymorphisms of *AKT1* rs1130233, *ICAM1* rs281432, *SELP* rs6128 and *TNSRSF1A* rs4149570, which have been recently suggested to be associated with the risk of cachexia/weight loss in Caucasian cancer patients [4], together with the *LIF* rs929271, and the risk of cancer cachexia as well as patients' prognosis in Japanese, leveraging the data from the cancer outpatient clinic of our hospital to find the way for the possible personalized palliative care of gastrointestinal patients based on genetic information.

Patients and Methods

Patients

Analysis of 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 till August 2015 was conducted. 53 patients underwent surgery and 6 did not. All of them underwent palliative chemotherapy and their body composition (such as total body weight, skeletal muscle weight and water weight) was measured mainly for the analysis of cachexia. All of the patients gave us written informed consent and provided clinical data and blood for the analysis and DNA testing. Patients' weight loss during 6 months after the initiation of chemotherapy was categorized as weight loss more than 5 percent (denoted as WL). Follow up of the patients' clinical information/conditions were conducted by checking up their electronic and/or paper medical records.

Genotyping

DNA was extracted from the patients' buffy coat, using the Qiagen Bio Robot EZ1 (Qiagen, Hilden, Germany). Genotypings of *LIF* rs929271, *AKT1* rs1130233, *ICAM1* rs281432, and *TNSRSF1A* rs4149570 were conducted by polymerase chain reaction with

confronting two-pair primers (PCR-CTPP) [9]. The primers used (and the thermal cycler conditions) for each SNP were as follows: F1: GAA CCA GCC CCC TGG AAG, R1: CCT TTC CCT GGT CCC TAC TCA A, F2: AGG GGC AGG GTT GTT CCA and R2: CGG GTG CCT TTC TGT CTT GA (initial denaturation at 95°C for 10 min, followed by 30 cycles of 95°C 1 min, 62°C 1 min and 72°C 1 min, and the final extension of 72°C 5 min) for *LIF* rs929271; F1: CCA CCT GTC CCG GGA A, R1: CGT GTG CTC AGG ACG TGG, F2: ATG CCT GCC CAG GCA G and R2: GTC CTC GGA GAA CAC ACG C (95°C for 10 min, followed by 30 cycles of 95°C 1 min, 60°C 1 min and 72°C 1 min, and the final extension of 72°C 5 min) for *AKT1* rs1130233; F1: ATA GGG AGT CAT GGA GGG TTT G, R1: CTT TAC CAA ATC CTG GTC ACT GAA, F2: AAA AAA TTG ATT GAT GGG AGG AAG and R2: TAA TCC CTG GCC TGC TCA G (95°C for 10 min, followed by 30 cycles of 95°C 1 min, 60°C 1 min and 72°C 1 min, and the final extension of 72°C 5 min) for *ICAM1* rs281432; F1: AAT TGG AAA ACA GAT CCA GAC AGT, R1: AGT GAG GCA GTG TTG CAA CAG, F2: CTT TGA GTT TTG GAT TGG ATC AGT and R2: AAT GAA CTT CTC AGA CAC ATA ACT GAA C (95°C for 10 min, followed by 30 cycles of 95°C 1 min, 59°C 1 min and 72°C 1 min, and the final extension of 72°C 5 min) for *TNSRSF1A* rs4149570 (the underline indicates the SNP base). We also genotyped *SELP* rs6128 by using the Taqman SNP Genotyping Assay (Applied Biosystems Co., Foster City, CA). Representative gels for genotyping are shown in Figure 1.

Statistical analysis

To evaluate survival time, Kaplan-Meier Curve, the logrank test, the Wilcoxon test and the Cox proportional hazard model were used. In addition, we analyzed the effect of genotype on patients' body weight by using the logistic regression model. The statistical software, STATA ver.13 (STATA Corp, TX), was used for analysis. This study

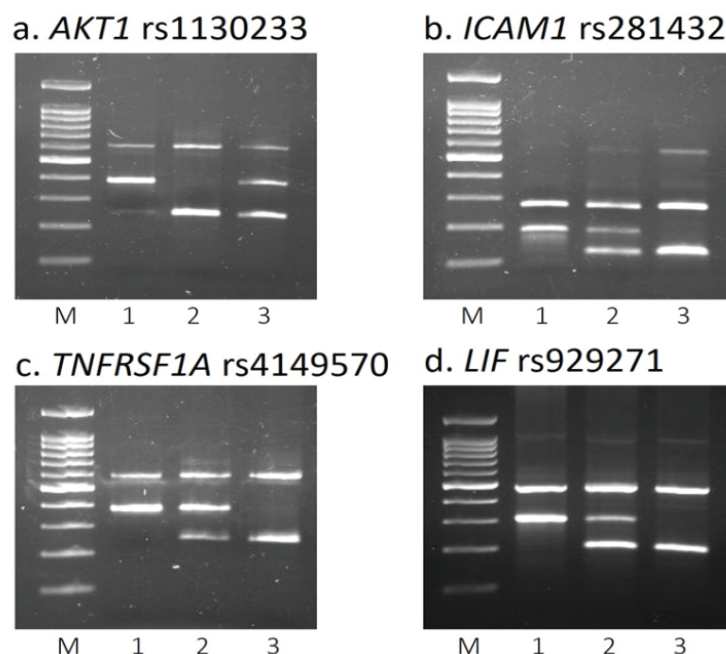


Figure 1: Representative gels for genotyping.

(a) *AKT1* rs1130233: Lane M, 100-bp marker; lane 1, A/A genotype (379-bp and 590-bp bands); lane 2, A/G genotype (245-bp, 379-bp and 590-bp bands); lane 3, G/G genotype (245-bp and 590-bp bands). (b) *ICAM1* rs281432: Lane M, 100-bp marker; lane 1, G/G genotype (186-bp and 272-bp bands); lane 2, G/C genotype (123-bp, 186-bp and 272-bp bands); lane 3, C/C genotype (123-bp and 272-bp bands). (c) *TNFRSF1A* rs4149570: Lane M, 100-bp marker; lane 1, T/T genotype (377-bp and 567-bp bands); lane 2, T/G genotype (244-bp, 377-bp and 567-bp bands); lane 3, G/G genotype (244-bp and 567-bp bands). (d) *LIF* rs929271: Lane M, 100-bp marker; lane 1, G/G genotype (291-bp and 473-bp bands); lane 2, T/G genotype (183-bp, 291-bp and 473-bp bands); lane 3, T/T genotype (183-bp and 473-bp bands).

was approved by the Institutional Review Board of Nagoya University Graduate School of Medicine (Approval no.2013-0220-08).

Results

Characteristics of patients are shown in Table 1. Table 2 shows the risk of weight loss (WL) with the crude OR (odds ratio), aOR-1 (sex- and age-adjusted odds ratio) and aOR-2 (sex- age- and clinical stage 4-adjusted odds ratio) by genotypes. A significant increase in the risk of WL in those with the A/G genotype of *AKT1* rs1130233 polymorphism (*AKT1* A/G vs. G/G, aOR-2=7.11; 95%CI: 1.41-35.7), or in those with at least one A allele of *AKT1* rs1130233 (*AKT1* A/G+A/A vs. G/G, aOR-2=4.57; 95% CI: 1.14-18.3) was observed. This association remained significant when the patients were restricted to those with colorectal cancers (*AKT1* A/G+A/A vs. G/G, aOR-2=6.59; 95% CI: 1.09-39.7) (Table 3). No other polymorphisms showed statistical significance.

Table 4 shows logrank P value and crude HR (hazard ratio) and the aHR (adjusted hazard ratio) for patients' survival. Kaplan-Meier Curves for the patients' survival by genotypes are shown in Figure 2. There was no statistically significant association of these 5 SNPs with patients' survival.

Variables	Values
Age [y (sd)]	68.1 (12.2)
Sex [n (%)]	
Male	37 (62.7)
Female	22 (37.3)
Cancer Type [n (%)]	
Esophageal	2 (3.4)
Stomach	11 (18.6)
Colorectal	40 (67.8)
Pancreatic	5 (8.5)
Biliary	1 (1.7)
UICC Stage [n (%)]	
I	5 (8.5)
II	10 (16.9)
III	15 (25.4)
IV	29 (49.2)
Genotype Frequency [n (%)]	
<i>SELP</i> rs6128	
A/A	14 (23.73)
A/G	31 (52.54)
G/G	14 (23.73)
<i>AKT1</i> rs1130233	
A/A	12 (20.34)
A/G	29 (49.15)
G/G	18 (30.51)
<i>ICAM1</i> rs281432	
C/C	28 (50.00)
G/C	22 (39.29)
G/G	6 (10.71)
<i>TNFRSF1A</i> rs4149570	
G/G	22 (37.29)
T/G	28 (47.46)
T/T	9 (15.25)
<i>LIF</i> rs929271	
G/G	8 (13.56)
G/T	32 (54.24)
T/T	19 (32.20)

Table 1: Characteristics of the study subjects.

SNP & genotype	WL (+)	WL (-)	crude OR	aOR-1	aOR-2
<i>SELP</i> rs6128					
A/A	8	3	1	1	1
A/G	15	12	0.46 (0.10-2.16)	0.45 (0.09-2.16)	0.71 (0.13-3.80)
G/G	9	4	0.84 (0.14-4.97)	0.82 (0.13-5.03)	1.38 (0.19-9.91)
<i>AKT1</i> rs1130233					
G/G	6	9	1	1	1
A/G	20	6	5 (1.26-19.8)	6.20 (1.42-27.0)	7.11 (1.41-35.7)
A/A	6	4	2.25 (0.43-11.5)	2.23 (0.42-11.8)	2.23 (0.39-12.6)
A/G+A/A	26	10	3.9 (1.10-13.8)	4.37 (1.17-16.2)	4.57 (1.14-18.3)
<i>ICAM1</i> rs281432					
C/C	13	10	1	1	1
G/C	15	5	2.2 (0.61-7.88)	2.15 (0.59-7.80)	2.41 (0.62-9.32)
G/G	2	3	0.48 (0.06-3.43)	0.50 (0.07-3.60)	0.76 (0.09-5.87)
<i>TNFRSF1A</i> rs4149570					
G/G	9	9	1	1	1
T/G	19	7	2.71 (0.76-9.63)	2.77 (0.70-10.9)	2.55 (0.60-10.7)
T/T	4	3	1.33 (0.22-7.74)	1.27 (0.20-8.02)	1.15 (0.16-7.97)
<i>LIF</i> rs929271					
T/T	10	5	1	1	1
G/T	18	11	0.81 (0.68-5.85)	0.82 (0.21-3.08)	0.87 (0.21-3.50)
G/G	4	3	0.66 (0.10-4.20)	0.69 (0.10-4.46)	0.72 (0.10-5.01)

WL (+) indicates the number of subjects with weight loss more than 5%; WL (-) indicates those with no weight loss. SNP: Single Nucleotide Polymorphism; OR: Odds Ratio (95% confidence interval in the parenthesis); aOR: Adjusted Odds Ratio (adjusted for age and sex); aOR-2: Adjusted Odds Ratio (adjusted for age, sex and UICC clinical stage 4)

Table 2: Risk of weight loss by genotypes.

SNP and genotype	WL (+)	WL (-)	crude OR	aOR-1	aOR-2
<i>AKT1</i> rs1130233					
G/G	4	8	1	1	1
A/G	11	4	5.49 (1.04-28.8)	5.45 (0.95-30.9)	6.47 (0.89-46.9)
A/A	4	2	4 (0.50-31.9)	4.13 (0.49-34.3)	6.79 (0.67-68.2)
A/G+A/A	15	6	5.0 (1.08-23.0)	4.97 (1.02-24.1)	6.59 (1.09-39.7)

WL (+) indicates the number of subjects with weight loss more than 5%; WL (-) indicates those with no weight loss. SNP: Single Nucleotide Polymorphism; OR: Odds Ratio (95% confidence interval in the parenthesis); aOR: Adjusted Odds Ratio (adjusted for age and sex); aOR-2: Adjusted Odds Ratio (adjusted for age, sex and UICC clinical stage4).

Table 3: Risk of weight loss by *AKT1* genotypes in colorectal cancer patients.

Discussion

There are a considerable number of studies on the relationship between SNPs and cancer risk or prognosis worldwide, but studies on the associations between SNPs and cachexia still remain scarce. One previous study suggested that *SELP* and *AKT1* polymorphisms may play roles in the risk of cachexia and death in pancreatic ductal adenocarcinoma patients (PDAC) in Caucasians [4]. In the present study, *SELP* and *AKT1* SNPs were analyzed and significant association was found on *AKT1*, where the risk of 5% weight loss in patients with

SNP	Comparison groups	Logrank P	Model	Crude HR	aHR-1	aHR-2
SELP rs6128	all 3 genotypes	0.79	additive	1.30 (0.56-3.02)	1.39 (0.59-3.26)	1.24 (0.51-3.00)
	vt. hetero+vt. homo vs. wt. homo	0.727	dominant	1.25 (0.34-4.52)	1.44 (0.37-5.49)	1.25 (0.33-4.75)
	vt. homo vs. others	0.508	recessive	1.55 (0.41-5.84)	1.68 (0.42-6.65)	1.48 (0.32-6.75)
AKT1 rs1130233	all 3 genotypes	0.488	additive	1.43 (0.65-3.14)	1.39 (0.52-3.72)	1.10 (0.42-2.88)
	vt. hetero+vt. homo vs. wt. homo	0.76	dominant	1.22 (0.33-4.55)	0.95 (0.21-4.16)	0.80 (0.185-3.50)
	vt. homo vs. others	0.232	recessive	1.96 (0.63-6.06)	2.05 (0.53-7.93)	1.52 (0.38-6.07)
ICAM1 rs281432	all 3 genotypes	0.461	additive	0.58 (0.24-1.39)	0.43 (0.15-1.22)	0.61 (0.19-1.96)
	vt. hetero+vt. homo vs. wt. homo	0.243	dominant	0.52 (0.17-1.58)	0.35 (0.09-1.27)	0.48 (0.12-1.85)
	vt. homo vs. others	0.43	recessive	0.04 (0.05-3.47)	0.41 (0.05-3.28)	1.29 (0.12-13.7)
TNFRSF1A rs4149570	all 3 genotypes	0.346	additive	1.66 (0.82-3.38)	2.06 (0.92-4.61)	1.85 (0.78-4.37)
	vt. hetero+vt. homo vs. wt. homo	0.173	dominant	2.37 (0.65-8.52)	2.49 (0.688-9.07)	1.82 (0.48-6.80)
	vt. homo vs. others	0.318	recessive	1.79 (0.55-5.76)	2.99 (0.80-11.1)	3.11 (0.78-12.3)
LIF rs929271	all 3 genotypes	0.585	additive	1.00 (0.45-2.22)	1.04 (0.47-2.31)	0.97 (0.43-2.17)
	vt. hetero+vt. homo vs. wt. homo	0.602	dominant	1.36 (0.42-4.38)	1.56 (0.47-5.17)	1.46 (0.43-4.86)
	vt. homo vs. others	0.479	recessive	0.48 (0.06-3.74)	0.43 (0.05-3.50)	0.37 (0.04-3.03)

SNP: Single Nucleotide Polymorphism; HR: Hazard Ratio; aHR: Adjusted Hazard Ratio (aHR-1, adjusted for sex and age; aHR-2, adjusted for age, sex and UICC clinical stage 4).

Table 4: Patient prognosis by genotypes.

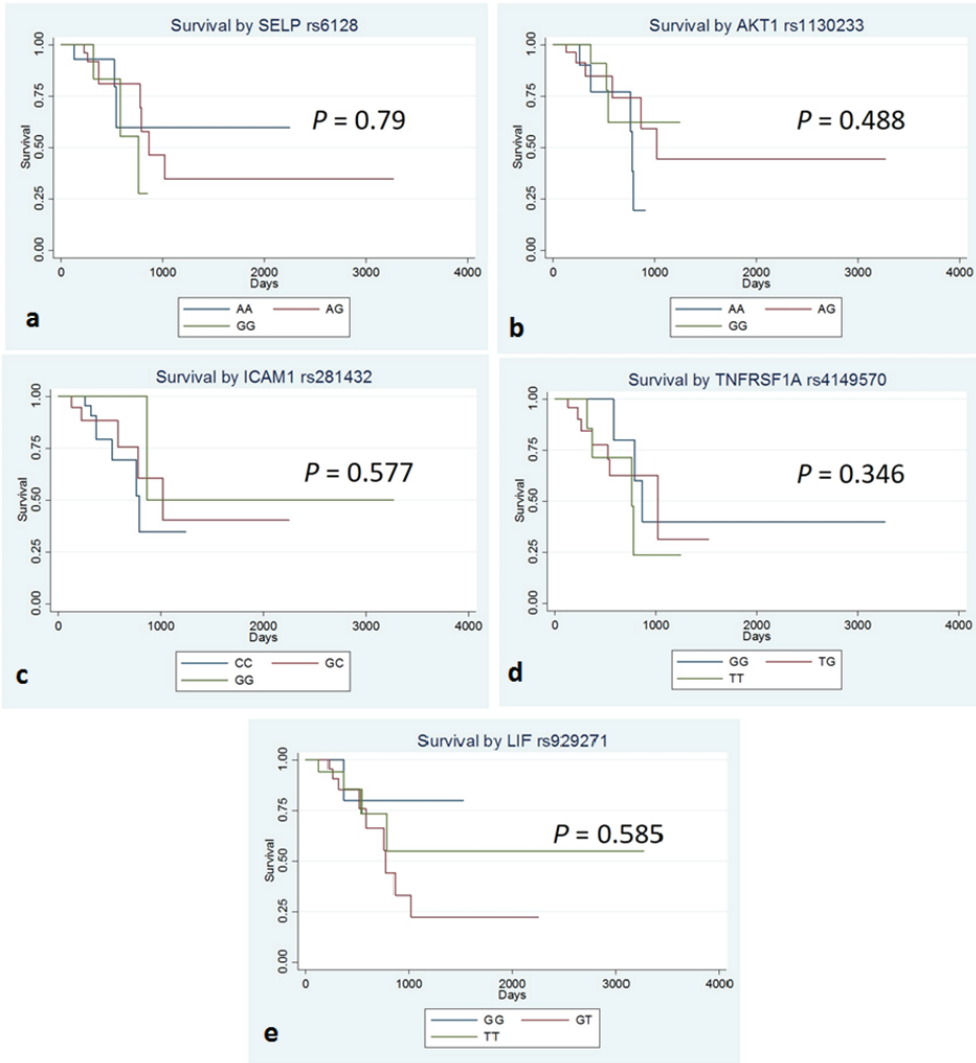


Figure 2: Survival curve by genotypes. a. Survival by *SELP* rs6128; b. Survival by *AKT1* rs1130233; c. Survival by *ICAM1* rs281432; d. Survival by *TNFRSF1A* rs4149570; e. Survival by *LIF* rs92927; P values indicate Logrank P values.

the *AKT1* A allele significantly increased. This finding was in the same trend as that reported by Avan et al. [4], suggesting that the *AKT1* A allele may play a key role in the development of cancer cachexia. There were no significant effects of the SNPs examined on patients' survival, presumably due to the relatively smaller sample size of the present study. *AKT1* responds to various stimuli such as hormones and growth factors, and it is involved in glucose metabolism, cell growth, and angiogenesis [10,11]. In cancer patients, the expression of *AKT1* increase, as cancer cells grow. *AKT1* is shown to exert its effects through various mediators, such as protein kinases and phosphatases, survival factors, regulators of protein synthesis, and so on, thereby play key roles in the development of cancer, cardiovascular diseases or metabolic diseases [12]. In human cancer cachexia, reduced activity of *AKT1* in those with *AKT1* rs1130233 G/A+A/A genotypes is considered to favor the induction of apoptosis, resulting in the higher risk of muscle atrophy and cachexia[4]. In the body of cachexia patients, production of inflammatory cytokine is induced, leading to the breakdown of fat and muscle. Given these biological facts reported, this reduced *AKT1* activity due to this *AKT1* SNP may lead to the development of severe weight loss in gastrointestinal cancer patients who suffer from tumor-induced inflammation. The allele frequencies of *AKT1* rs1130233 differ by race, with the A allele frequency of 0.300 in Caucasians, 0.051 in Africans and 0.575 in East Asians (consisting of Japanese and Chinese) according to dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP>), suggesting the possible existence of population specific susceptibility to cancer cachexia. The roles of *AKT1* in human cancer cachexia in various races/ethnicities need to be further verified in the near future.

The other functional SNPs in *SELP*, *ICAM*, *TNRSF1A* and *LIF* genes did not show any statistical significance, suggesting the limited roles of these SNPs in cachexia of gastrointestinal cancer patients. The data in this study was gathered once or twice a week, making it a minute and detailed data. The number of subjects in the present study was 59, the statistical power of which was 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 is 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 is more than 55% for an HR of 2 or 0.5 under the same conditions. Although the present study could not reproduce the previously reported association of *AKT1* SNP with risk of weight loss in pancreatic cancer patients, it revealed the novel association of the corresponding *AKT1* rs1130233 SNP with weight loss in colorectal cancer patients as well as gastrointestinal cancer patients as a whole, suggesting the possible feasibility and usefulness of examining this *AKT1* SNP in gastrointestinal cancer patients under palliative care.

Conclusions

The present study suggested the possibly important role of *AKT1* in the development of cachexia in gastrointestinal cancer patients. Further investigation of the role of this *AKT1* SNP in the context of gastrointestinal cancer palliative care, as well as those with regard to the biological roles of *AKT1* in human cancer cachexia would help better understand the potential roles of this molecule in the near future.

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Conflict-of-interest statement

The authors declare that they have no financial conflicts of interest to disclose.

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