Cancer cachexia is associated with the *IL10 −1082* gene promoter polymorphism in patients with gastroesophageal malignancy[1–3]

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ABSTRACT

Background: The genetic predisposition of the host to local or systemic inflammation may contribute to the effect of cancer cachexia.

Objective: We investigated the relation between cytokine polymorphisms (*IL1B −511, IL6 −174, IL10 −1082, TNFA −308,* and *LTA +252*) and markers of nutritional status among patients with gastroesophageal cancer to determine whether any such association was reflected by cytokine concentrations in the tumor or plasma compartments.

Design: Patients ($n = 203$) with a diagnosis of gastroesophageal cancer underwent nutritional assessment (body mass index, anthropometric measures, dysphagia scoring, and estimation of dietary intake). Single nucleotide polymorphism genotyping was performed by TaqMan allelic discrimination genotyping. Serum cytokine and C-reactive protein concentrations were determined by enzyme-linked immunosorbent assay. Tumor tissue cytokine protein concentrations ($n = 56$) were determined by using the Cytometric Bead Array System.

Results: *IL10 GG* and *IL6 CC* polymorphisms were associated with elevated serum C-reactive protein concentrations, and the *IL6 CC* genotype was also associated with elevated tumor tissue cytokine concentrations. At diagnosis, the *IL10 GG*, but not the *IL6*, genotype was linked with increased total weight loss: 4.9% for *AA*, 7.1% for *AG*, and 12.0% for *GG* ($P = 0.007$). Serum C-reactive protein concentrations correlated with increased weight loss ($r = 0.24$, $P < 0.001$). Compared with other genotypes, the *IL10 GG* genotype retained an independent association in determining the extent of weight loss on multivariate analysis (95% CI: 0.52, 3.43; $P = 0.008$). Possession of the *GG* allele was associated with a 2.3 times increased risk of developing cachexia (95% CI: 1.2, 4.3; $P = 0.014$).

Conclusion: These data suggest that the *IL10* genotype of the host can influence the development of cachexia among patients with gastroesophageal malignancy. *Am J Clin Nutr* 2009;89:1164–72.

INTRODUCTION

Systemic inflammation is commonly associated with lung, gastrointestinal, and ovarian malignancy and has been linked with adverse effects on survival (1–4). The association between systemic inflammation and adverse outcome is explained by tumor-related mechanisms, including enhanced tumor progression, angiogenesis, and metastatic potential. Alternatively, systemic inflammation is associated with weight loss, hypermetabolism, and reduced food intake (5–7). These components of the cachexia syndrome also result in shortened survival (8). The link between systemic inflammation, cancer, and the cachexia syndrome remains incompletely understood, but it is likely that proinflammatory cytokines play an important role (9, 10).

Weight loss is commonly associated with gastroesophageal malignancy and is assumed to be secondary to the mechanical effects of the tumor on the upper digestive tract. Obstruction to swallowing, early satiety, nausea, and vomiting are common symptoms. However, weight loss is frequent among patients with relatively few obstructive symptoms, and cachexia is also common among patients with tumors not directly involving the gastrointestinal tract (1, 2). Weight loss in gastroesophageal malignancy is therefore likely to be multifactorial.

Many proinflammatory cytokine genotypes have been linked with differential levels of cytokine expression, which, in turn, have been shown to be associated with systemic inflammation in the host (11–13). In previous work, our group identified a link between *IL6* and *IL10* promoter polymorphisms and elevated markers of systemic inflammation in the serum of patients with gastroesophageal malignancy (14). These genotypes were also linked with adverse prognosis, presumably through systemic inflammation.

Host cytokine genotype can influence the ability of tumors to produce proinflammatory cytokines. Martignoni et al (15) identified tumor interleukin-6 (IL-6) overexpression with an ability to sensitize peripheral blood mononuclear cells and in-

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duce further IL-6 expression with weight loss in patients with pancreatic cancer. We showed previously that tumor tissue IL-1β overexpression is associated with systemic inflammation and poor outcome in gastroesophageal malignancy (16).

The genotype of the host cytokine can influence cachexia through altered levels of cytokine production within the tumor and the host. An association between an IL-1β single nucleotide polymorphism (SNP) and increased weight loss among patients with gastric and esophageal cancer has been reported (17, 18). The *IL1B − 511* gene promoter polymorphism has also been implicated in cachexia associated with pulmonary disease (19). Similarly, the *IL6 –174* genotype has been linked with resistance to weight gain among patients with energy starvation (20). Host cytokine genotype may influence weight loss in cancer and contribute to accelerated demise.

The aim of this study was to investigate the relation between cytokine polymorphisms (*IL1B −511*, *IL6 −174*, *IL10 −1082*, *TNFA −308*, and *LTA +252*) and markers of nutritional status among patients with newly diagnosed gastroesophageal cancer and to determine whether any such association is reflected by cytokine concentrations in the tumor or plasma compartments.

SUBJECTS AND METHODS

Study population

All patients with gastric or esophageal cancer within the Lothian and Borders regions, Scotland, between March 2002 and June 2004 were eligible for inclusion in the study. No patients were excluded. All subjects provided written informed consent, and the study received ethical permission from the Lothian Research Ethics Committee. Every patient underwent an assessment of their nutritional status at the time of recruitment and blood was collected at the same time for determination of cytokine genotype and measurement of serum cytokine and CRP concentrations. Samples of tumor tissue were collected from patients who underwent surgical resection for analysis of tumor tissue cytokine concentrations. Patients were staged as per our unit protocol and, after discussion at multidisciplinary team meetings, underwent various treatment options.

Nutritional assessment

All patients underwent an assessment of their nutritional status at the time of recruitment to the study. This involved calculation of body mass index (BMI; in kg/m^2) and anthropometric measurements. BMI was calculated after measurement of the patients' height and weight. Premorbid weight was recalled by the patient and verified where possible from the medical notes. Individual weight loss was calculated and expressed as percentage of pre-illness body weight lost. Rate of weight loss was expressed as percentage body weight lost per month of symptoms. Anthropometric measurements included measurement of midarm circumference (MAC) and triceps skinfold thickness. MAC was measured at the midpoint between the acromion and the olecranon processes. Triceps skinfold thickness was measured with Harpenden skin calipers (Holtain, Byberian, United Kingdom), and midarm muscle circumference (AMC) was calculated by means of Jelliffe's equation (21). Values were normalized by using standardized reference tables (22).

Dietary intake was estimated by all patients and scored as normal, reduced, or poor/minimal. This simple assessment of dietary intake was validated in a subgroup of patients by using detailed food diaries. Patients ($n = 22$) were instructed to record all food and drink consumed over a 3-d period, and the data were analyzed by a senior dietitian who provided information on the level of intake of macronutrients by using computer software (CompEat; Nutrition Systems, Grantham, United Kingdom). Comparisons were made to dietary reference values issued by the Department of Health (23). The severity of dysphagia was assessed by interview and was scored according to Knyrim et al (24).

Cytokine genotyping

Genomic DNA was extracted from samples of lithium-heparinized blood by using the Wizard Genomic DNA purification kit (Promega, Southampton, United Kingdom). The following SNPs were selected for genotyping: *IL1B −511, IL6–174, IL10–1082, TNFA −308,* and *LTA +252*. Genotyping was carried out by TaqMan allelic discrimination genotyping on the 7900HT Sequence Detection System (Applied Biosystems, Warrington, United Kingdom) as described in detail previously (14). Briefly, 10-μL polymerase chain reactions containing 20 ng DNA and 0.9- and 0.2-μmol/L probes (final concentrations) were performed in 384-well plates. Each genotyping plate contained no DNA template (water) controls; SDS version 2.1 software (Applied Biosystems, Warrington, United Kingdom) was used to analyze real-time and endpoint fluorescence. Approximately 50 samples ($\approx$25% of the sample group) were randomly selected and included as replicates for each genotype tested. All replicates agreed. Only between 3 and 8 samples failed to genotype for each SNP (96.1–98.5% completion rate).

Determination of serum cytokine and CRP concentrations

Blood was collected from every patient at the time of diagnosis and before any therapeutic intervention. Serum CRP concentration was measured with an automated immunoturbidimetric assay within the Clinical Biochemistry Department, Edinburgh Royal Infirmary, United Kingdom. With this assay, a concentration of 10 mg/L represents the upper limit of the normal range, with most healthy individuals having a serum concentration <2 mg/L (25). The CV was $<3\%$.

Cytokine concentrations were determined by sandwich enzyme-linked immunosorbent assay with module kits and performed according to the manufacturer's instructions (Caltag; Bender MedSystems, Towcester, United Kingdom) as described previously (26). The lower limit of sensitivity for each assay was as follows: <1 pg/mL IL-1β, 1.4 pg/mL IL-6, 0.8 pg/mL IL-10, and 5.8 pg/mL soluble tumor necrosis factor receptor (sTNF-r).

Measurement of tumor tissue cytokine protein concentrations

Tissue was obtained from 56 patients at the time of surgical resection. Representative samples of tumor tissue were stored at -80°C until batch analysis. Tissue samples were homogenized and cytokine protein concentrations were determined by using the Cytometric Bead Array System (Human Inflammation Kit; BD Biosciences, Oxford, United Kingdom) as published previously (16). This kit allows the simultaneous measurement of cytokines

IL-1β, IL-6, IL-8, IL-10, IL-12p70, and tumor necrosis factor-α (TNF-α). Briefly, 50 μL of tissue extract was added to the reaction mix containing antibody-coated microbeads and incubated at room temperature for 3 h Cytokine concentrations were determined by flow cytometry (FACScan; BD Biosciences). Results were calculated to take into account the total protein concentration of the tissue lysate and are expressed as pg/mg of total protein. Intraassay variability ranged between 2% and 10% and interassay variability was 4–15%.

Statistical analysis

Correlations were investigated by Spearman's rank test. Independent variables were analyzed with the Mann-Whitney *U* or the Kruskal-Wallis tests. Significant *P* values were corrected for multiple comparisons by using Bonferroni correction. Categorical data were analyzed with the chi-square test. Multiple regression modeling was used to identify which variables were independently associated with weight. Data analysis was performed by using SPSS software (version 11; SPSS, Chicago, IL).

RESULTS

Study patients and genotype distributions

Demographic characteristics of the patients are shown in **Table 1**. The 203 patients were genotyped; the primary tumor sites were esophageal ($n = 91$; 45%), gastric ($n = 75$; 37%), and those arising from the gastroesophageal junction ($n = 37$; 18%).

TABLE 1
Demographic characteristics of the study patients ($n = 203$)[1]

	Value
Age (y)	71 (62–78)[2]
Sex [*n* (%)]	
Female	69 (34)
Male	134 (66)
Tumor site [*n* (%)]	
Esophageal	91 (45)
Esophagogastric junction	37 (18)
Gastric	75 (37)
Histology [*n* (%)]	
Adenocarcinoma	172 (85)
Squamous cell carcinoma	26 (13)
Indeterminate	5 (2)
Grade [*n* (%)]	
Well differentiated	5 (2)
Moderately differentiated	69 (34)
Poorly differentiated	129 (64)
UICC stage [*n* (%)]	
1	23 (12)
2	33 (16)
3	76 (37)
4	71 (35)
Treatment [*n* (%)]	
Surgery with curative intent	91 (5)
Preoperative chemotherapy/surgery	22 (11)
Chemoradiotherapy with curative intent	7 (3)
Palliative chemotherapy	24 (12)
Palliative radiotherapy	6 (3)
Stent/dilatation/laser/symptomatic	53 (26)

[1] UICC, International Union against Cancer.
[2] Value is median; interquartile range in parentheses.

Histologic confirmation of disease was obtained in all cases, and the predominant histologic subtype was adenocarcinoma (85%). One hundred thirteen (56%) patients underwent surgical resection, 22 of whom received preoperative chemotherapy. Seven (3%) patients, all of whom had squamous cell carcinoma of the esophagus, received chemoirradiation with curative intent. The remaining patients ($n = 90$) were not suitable for curative therapy and received palliative chemotherapy/radiotherapy or underwent alternative palliative treatments, such as insertion of a stent or endoscopic laser therapy. The allelic distribution of cytokine genotypes among the study population is shown in **Table 2**. All genotype frequencies were distributed in accordance with the Hardy-Weinberg equilibrium, except for *IL6 –174* ($P = 0.05$).

Assessment of nutritional status

Patients had lost a median of 7.1% (interquartile range: 1.1–13.9%) total body weight at the time of diagnosis compared with their premorbid stable body weight. This was equivalent to a median rate of weight loss of 3.2% total body weight per month of illness (interquartile range: 0.5–6.3%/mo), defined from the onset of symptoms. Seventy-nine (39%) patients remained weight steady at the time of diagnosis (weight loss <5%), 44 (22%) patients had lost between 5% and 10% of body weight, and the remaining 80 (39%) patients had lost >10% of their pre-illness body weight at the time of diagnosis. Anthropometric values were compared with published national normalized values, matched for age and sex (22). Compared with values published for a healthy population, the study patient's measurements were reduced for MAC, median percentile group = 10 (interquartile range: 1st–25th percentile); triceps skinfold, median percentile group = 25 (interquartile range: 10th–50th percentile); and AMC, median percentile group = 5 (interquartile range: 1st–25th percentile).

Assessment of dietary intake and dysphagia score

Seventy-eight (38%) patients described their dietary intake as normal, 94 (46%) as reduced compared with normal, and 31 (15%) as poor or minimal at diagnosis. Detailed 3-d food diaries were collected from 22 (11%) patients. Actual food intake was compared with perceived food intake for those patients who completed food diaries. Patient perception of reduced food intake was associated with a reduced total calorie intake; "normal" intake (median: 90% of the Estimate Average Requirement in kcal; interquartile range: 81–105) compared with "reduced/poor" intake (median: 76% of the Estimate Average Requirement in kcal; interquartile range: 56–89) ($P = 0.040$, Mann-Whitney *U* test) and reduced protein intake (normal intake median: 170% of the Reference Nutrient Intakes in g/d; interquartile range: 133–197) compared with reduced/poor intake (median: 113% of the Reference Nutrient Intakes in g/d; interquartile range: 99–148) ($P = 0.003$).

Eighty-five (42%) patients had no dysphagia at diagnosis, 85 (42%) were able to swallow solids or semisolid foods, 30 (15%) were able to swallow liquids only, and 3 (1%) had total dysphagia. Esophageal tumors were associated with increased dysphagia scores ($P < 0.001$; chi-square test), but there was no difference in the overall dietary intakes between the 3 tumor groups ($P = 0.383$) (**Table 3**).

TABLE 2
Allelic distribution of cytokine genotypes among the study population

Cytokine genotype and allele	Study patients
	n (%)
IL1B −511	
CC	89 (45.4)
CT	81 (41.3)
TT	26 (13.3)
P[1]	0.27
IL6 –174	
GG	71 (36.0)
GC	83 (42.1)
CC	43 (21.8)
P[1]	0.05
IL10 –1082	
GG	54 (27.0)
AG	93 (46.5)
AA	53 (26.5)
P[1]	0.32
TNFA −308	
GG	124 (62.0)
AG	61 (30.5)
AA	15 (7.5)
P[1]	0.06
LTA +252	
AA	82 (42.1)
AG	84 (43.1)
GG	29 (14.9)
P[1]	0.33

[1] *P* value for Hardy-Weinberg equilibrium (chi-square test).

TABLE 3
Nutritional intake and dysphagia score stratified according to primary tumor site

	Tumor site			
	Esophageal ($n = 91$)	Esophagogastric junction ($n = 37$)	Gastric ($n = 75$)	*P* value[1]
Dietary intake				0.383
Normal	33	12	32	
Reduced	45	21	28	
Poor/minimal	13	4	15	
Dysphagia score				<0.001
0	16	7	62	
1	28	13	1	
2	26	12	5	
3	20	4	6	
4	1	1	1	

[1] Chi-square test.

Link between weight loss and serum cytokine and serum CRP concentrations

The median serum CRP concentration measured at the time of diagnosis was 7 mg/L (interquartile range: 2–24 mg/L). Elevated CRP concentrations correlated positively with increased total weight loss ($r = 0.24$, $P < 0.001$; Spearman rank analysis) and rate of weight loss ($r = 0.23$, $P < 0.001$).

IL-1β and IL-10 were detected in only 4 (2%) and 10 (5%) of the patient's serum, respectively. Given the low level of expression, IL-1β and IL-10 were excluded from subsequent analysis. Elevated serum IL-8 and sTNF-r concentrations correlated with increased total weight loss ($P = 0.020$, $r = 0.17$ and $P = 0.041$, $r = 0.15$ respectively; Spearman rank analysis), Serum IL-6 concentrations did not correlate with any nutritional variable.

Link between genotype and serum cytokine and CRP concentrations

The *IL10 GG* genotype was associated with elevated serum CRP concentrations (median: 16 mg/L; range: 3–44 mg/L) when compared with the *AA* genotype (median: 6 mg/L; range: 3–13 mg/L) ($P = 0.016$, Mann-Whitney *U* test) and the *AG* genotype (median: 5 mg/L; range: 2–24 mg/L) ($P = 0.033$). The *GG* genotype was also associated with elevated sTNF-r concentrations when compared with the *AG* genotype ($P = 0.05$), but not when compared with *AA* homozygotes ($P = 0.22$).

The *IL6 CC* homozygous genotype was associated with elevated serum CRP concentrations when compared with the *GG* (median: 13 mg/L; range: 4–35 mg/L) and *GC* (median: 6 mg/L; range: 2–22 mg/L) genotypes ($P = 0.032$, Mann-Whitney *U* test). *IL6 CC* genotype was also associated with elevated serum sTNF-r concentrations when compared with the *GC* genotype ($P = 0.015$, Mann-Whitney *U* test), but not when compared with the *GG* genotype ($P = 0.14$). *IL1B −511, TNFA −308*, and *LTA +252* genotypes were not associated with serum cytokine or CRP concentrations.

Genotype and tumor tissue cytokine concentrations

The *IL6* genotype was linked with tumor tissue cytokine concentrations. The association between individual *IL6* genotypes and tumor cytokine concentrations are presented in **Table 4**. There was a trend for the *IL6 CC* genotype to be linked with elevated tumor cytokine concentrations when compared with the other individual genotypes (Table 4). However, given the relatively small numbers involved in each subgroup and to test whether the *CC* allele was a risk factor for elevated cytokine concentrations, the *GC* and *GG* genotypes were combined and were analyzed as a single group compared with the *CC* genotype. After the revised analysis, the *IL6 CC* genotype was associated with elevated tumor tissue IL-6 ($P = 0.029$, Mann-Whitney *U* test), IL-10 ($P = 0.009$), and IL-12 ($P = 0.012$) protein concentrations (**Figure 1**). There was also a trend for elevated tissue TNF-α concentrations, but it was not significant ($P = 0.08$). Additionally, the *IL1*β *AA* genotype was linked with reduced tumor IL-8 concentrations when compared with the *GG* genotype ($P = 0.021$) and the *AG* genotype ($P = 0.042$). None of the other genotypes were associated with tissue cytokine protein concentrations.

Link between genotype and markers of nutritional status

The *IL10* genotype was associated with increased total weight loss at the time of diagnosis and with accelerated rates of weight loss: *AA* genotype (median weight loss: 4.9%; interquartile range: 0–10.2%), *AG* genotype (median weight loss: 7.1%; interquartile range: 1.1–13.9%), and *GG* genotype (median weight loss: 12.0%; interquartile range: 3.3–16.8%) ($P = 0.007$, Kruskal-Wallis test) (**Figure 2** and **Table 5**). Significant *P* values were corrected for multiple comparisons by using Bonferroni

TABLE 4
Tissue cytokine concentrations measured in homogenized gastroesophageal tumor tissue stratified according to *IL6 –174* genotype[1]

	Genotype group				
Tissue cytokine proteins	Whole group ($n = 43$)	*CC* ($n = 24$)	*CG* ($n = 6$)	*GG* ($n = 13$)	*P* value[2]
	pg/mg total protein				
IL-1β	135.8 (40.5–424.7)	270 (121–298)	125 (30–580)	104 (36–373)	0.587
IL-6	3.0 (0–45.8)	50 (9–79)	0 (0–91)	3 (0–8)	0.191
IL-8	56.2 (22.6–158.6)	106 (22–164)	39 (14–259)	76 (29–116)	0.740
IL-10	12.5 (0–101.1)	115 (31–158)	2 (0–225)	10 (0–46)	0.083
IL-12	7.6 (0–67.3)	67 (25–146)	2 (0–161)	2 (0–33)	0.077
TNF-α	6.6 (1.1–25.8)	18 (13–27)	5 (0–37)	4 (1–21)	0.267

[1] All values are medians; interquartile ranges in parentheses. IL, interleukin; TNF-α, tumor necrosis factor-α.
[2] Kruskal-Wallis test.

correction. Significant differences remained between the *GG* and *AA* genotypes for total weight loss ($P = 0.004$) and rate of weight loss ($P = 0.005$) (Table 5). There were no differences in dietary intakes between the different genotype groups, either in those patients who completed detailed food diaries or in those who estimated their dietary intake. In addition, patients in each genotype subgroup had similar dysphagia scores ($P = 0.236$, chi-square test). The *IL1B −511, IL-6–174, TNFA −308*, and *LTA +252* genotypes were not associated with any nutritional variables.

Multivariate analysis

Multiple regression analysis was performed to identify whether the *IL10* genotype retained association with weight loss, independent of systemic inflammation and other covariates. Level

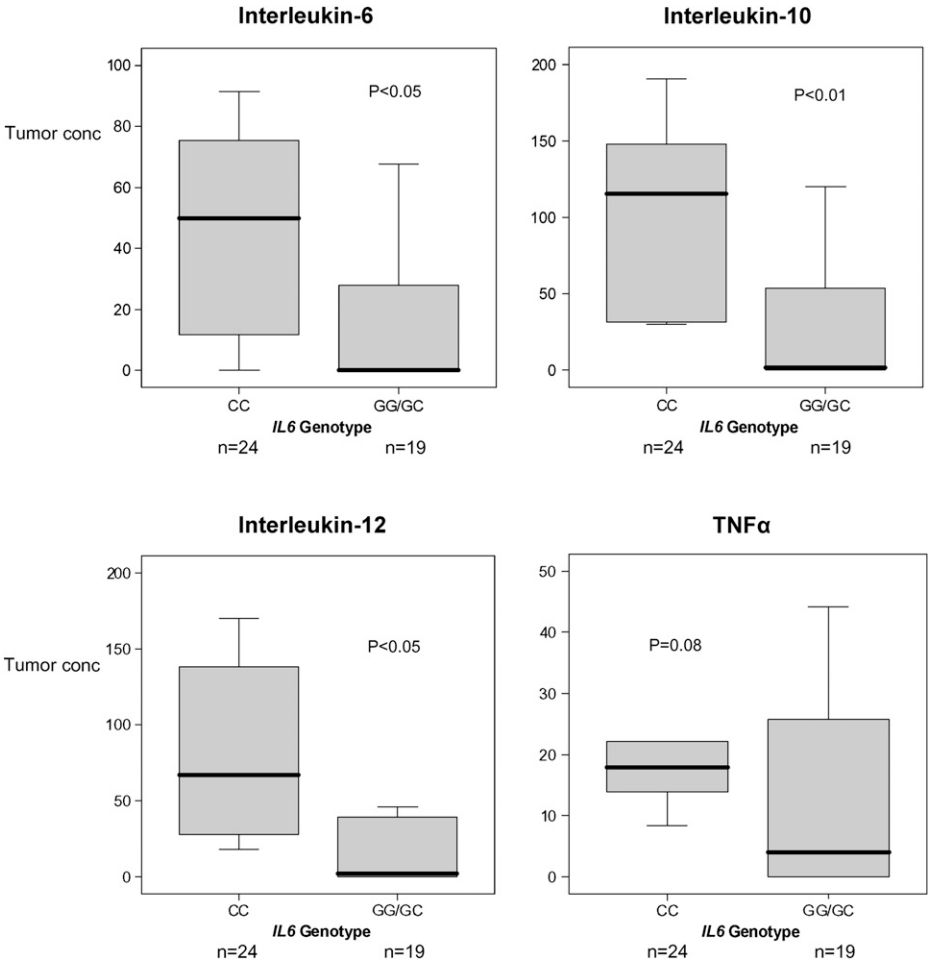

FIGURE 1. The interleukin-6 (*IL6*) *CC* genotype was associated with elevated tumor tissue cytokine concentrations (conc) when compared with the *CG/GG* genotypes. Boxes show interquartile ranges; horizontal lines shows medians. The error bars represent extreme cases. IL-6 ($P = 0.029$), IL-10 ($P = 0.009$), IL-12 ($P = 0.012$), and tumor necrosis factor-α (TNFα); $P = 0.08$, Mann-Whitney *U* test.

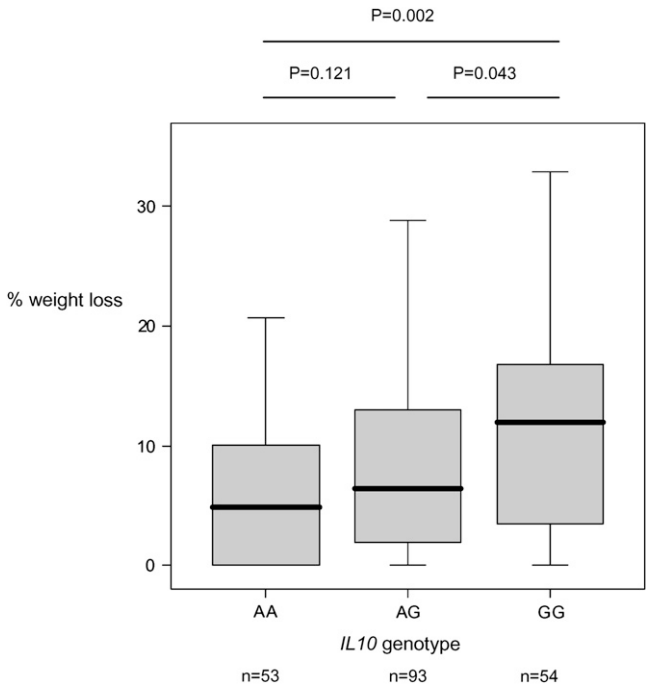

FIGURE 2. The interleukin-10 (*IL10*) genotype was associated with increased total weight loss at the time of diagnosis. *AA* genotype (median weight loss: 4.9%; interquartile range: 0–10.2%), *AG* genotype (median weight loss: 7.1%; interquartile range: 1.1–13.9%), and *GG* genotype (median weight loss: 12.0%; interquartile range: 3.3–16.8%). Boxes show interquartile ranges; horizontal lines shows medians. The error bars represent extreme cases. The Mann-Whitney *U* test was used for statistical analysis.

of dietary intake, serum CRP concentration, stage of disease, and dysphagia score were associated with weight loss and were chosen, in addition to *IL10* genotype, for inclusion in the multivariate analysis. Dysphagia score and level of dietary intake are clearly confounding variables and, therefore, dietary intake alone was selected for inclusion in the model. Dietary intake (hazard ratio: 3.5; 95% CI: 1.82, 5.11; $P < 0.001$), stage of disease (hazard ratio: 1.4; 95% CI: 0.26, 2.58; $P = 0.017$), and *IL10* genotype (hazard ratio: 2.0; 95% CI: 0.52, 3.43; $P = 0.008$) all retained independent association in determining the extent of weight loss (**Table 6**). Assuming a definition of cachexia as weight loss >10% premorbid weight, when compared with the *IL10 AA* genotype, possession of the *AG* genotype was associated with a hazard ratio of 1.3 (95% CI: 1.12, 1.94; $P = 0.019$; Cox's univariate model) for the development of cachexia and possession of the *GG* genotype was associated with a hazard ratio of 2.3 (95% CI: 1.18, 4.30; $P = 0.014$) (**Table 7**).

DISCUSSION

In the present study, the *IL10 –1082* polymorphism was associated with increased total weight loss and rates of weight loss among patients with gastroesophageal malignancy at the time of diagnosis, independent of dietary intake, stage of disease, and serum CRP concentrations. Furthermore, possession of the *GG* genotype was associated with a 2.3 times increased risk of developing cachexia.

Weight loss at diagnosis was commonly observed within the study population, with 61% of patients experiencing weight loss to any degree and 39% ($n = 80$) of patients losing ≥10% of their pre-illness body weight. In the present study, we also found reduced anthropometric measurements: median MAC and AMC values were in the 10th percentile, and triceps skinfold thickness values were in the 25th percentile. Values below the 15th percentile are generally representative of some degree of nutritional depletion, and these data may suggest a preferential loss of lean body mass rather than fat mass as is characteristic of the cachexia syndrome (22).

There was a positive relation between elevated markers of systemic inflammation and markers of nutritional depletion. Serum CRP and sTNF-r concentrations were positively correlated significantly with weight loss, a finding in keeping with previous studies on human cancer subjects (6, 7). Serum cytokine concentrations were generally not associated with any nutritional variable. IL-8 concentrations were correlated with increased total weight loss, but not with rate of weight loss or any other nutritional variable. Elevated IL-8 concentrations were previously observed in the serum of patients with pancreatic cancer and are involved in the inflammatory cascade that may contribute to systemic inflammation and weight loss (11). Although other groups have shown associations between serum cytokine concentrations and markers of nutritional depletion, we found no such association in this study or previously in a study of patients with pancreatic cancer (7). The difficulties in reliably measuring circulating cytokine concentrations have been documented extensively, and the relevance of such measurements must also be considered. Local tissue cytokine production by inflammatory cells (or tumor cells) is likely to be a better indicator of cytokine activity in cancer cachexia (7, 15). However, circulating acute phase proteins (eg, CRP) remain robust indexes of systemic proinflammatory status.

Possession of the *IL10 –1082 G* allele was associated with increased weight loss. Significantly increased rates of weight loss were observed among patients possessing both *GG* alleles. The *IL10* polymorphism at position –1082 lies within the promoter region of the gene, and the *GG* genotype has been shown to be associated with increased IL-10 production (11, 27). In acute inflammatory states, such as gram-negative sepsis, the *IL10 GG* genotype has been shown to be associated with a higher risk of developing septic shock and with poorer outcomes (27). In chronic inflammatory conditions, such as cancer, including among patients with gastroesophageal malignancy, possession of the *GG* genotype has also been linked with adverse outcome (14, 28). The link between cytokine genotype and accelerated demise may be explained in part through the association between genotype and the generation of the systemic inflammatory response. However, among cancer patients, such an association may also be explained by accelerated weight loss and the development of the cachexia syndrome. In animal studies, elevated serum IL-10 concentrations have been measured in malnourished mice compared with healthy mice (29). In human studies, significantly lower serum IL-10 concentrations have been recorded in obese women than in healthy controls (30). Serum IL-10 concentrations have been shown to be positively correlated with weight loss in patients with pancreatic cancer (31). It is possible, therefore, that elevated circulating concentrations of IL-10 are linked with increased rates of weight loss in cancer patients. However, serum IL-10 was only detectable in 10 patients in the current study, which makes such conclusions difficult to confirm.

TABLE 5
Nutritional variables for the patient group measured at the time of diagnosis, stratified by *IL10* genotype[1]

	Genotype			
	AA ($n = 53$)	*AG* ($n = 93$)	*GG* ($n = 54$)	*P* value[2]
Pre-illness BMI (kg/m^2)	26.6 (23.1–30.6)	26.4 (23.9–30.1)	26.3 (24.4–30.2)	0.959
BMI at diagnosis (kg/m^2)	25.6 (21.7–28.7)	24.6 (21.2–27.9)	23.9 (20.7–27.4)	0.214
Total body weight loss (%)	4.9 (0–10.2)	7.1 (1.1–13.9)	12.0 (3.3–16.8)	0.007
AA vs *AG*	—	—	—	0.121 (0.344)[3]
GG vs *AA*	—	—	—	0.002 (0.004)[3]
GG vs *AG*	—	—	—	0.043 (0.122)[3]
Rate of weight loss (%/mo)	2.2 (0–4.6)	3.2 (0.5–6.3)	5.4 (1.5–7.6)	0.008
AA vs *AG*	—	—	—	0.133 (0.280)[3]
GG vs *AA*	—	—	—	0.002 (0.005)[3]
GG vs *AG*	—	—	—	0.046 (0.159)[3]
Midarm circumference (percentile group)	10 (5–25)	10 (1–25)	10 (1–25)	0.347
Triceps skinfold thickness (percentile group)	25 (25–50)	25 (10–50)	25 (5–50)	0.186
Arm muscle circumference (percentile group)	5 (1–25)	5 (1–25)	10 (1–50)	0.748
Food diary intake[4]	—	—	—	—
Energy kcal (% of EAR)	65 (56–91)	87 (68–93)	82 (64–104)	0.116
Protein (% of RNI)	115 (104–192)	142 (109–170)	129 (97–169)	0.325
Dietary intake [*n* (%)]	—	—	—	0.123[5]
Normal	23 (44)	35 (38)	13 (24)	—
Reduced	21 (40)	44 (47)	32 (59)	—
Poor/minimal	9 (16)	14 (15)	9 (18)	—
Dysphagia score [*n* (%)]	—	—	—	0.236[5]
0	22 (42)	39 (42)	24 (45)	—
1	15 (28)	19 (21)	6 (12)	—
2	11 (20)	19 (21)	10 (18)	—
3	5 (10)	14 (15)	13 (24)	—
4	0	2 (2)	1 (2)	—

[1] Values are medians; interquartile ranges in parentheses (unless otherwise noted). EAR, Estimated Average Requirement; RNI, Reference Nutrient Intake.
[2] Kruskal-Wallis test, except where otherwise noted. Significant *P* values were corrected for multiple comparisons by using Bonferroni correction (values in parentheses).
[3] Mann-Whitney *U* test.
[4] Calculated from a subgroup of 22 patients.
[5] Chi-square test.

In contrast, a recent animal model for gastric cancer has suggested a survival benefit associated with elevated peritoneal IL-10 concentrations (32). In that study, adenoviral gene transfer was used to increase peritoneal IL-10 concentrations in a mouse peritoneal dissemination model of gastric cancer. The authors reported reduced tumor bulk, reduced angiogenesis, and improved cachexia associated with elevated IL-10 concentrations, which resulted in overall better survival. These apparent contradictions may be explained by different effects of IL-10 in different body compartments. Local IL-10, for example, within the peritoneal cavity, can induce a localized immunological response through lymphocyte activation, which results in a tumor-mediated cytotoxic response (33, 34). Conversely, the overall systemic consequences of elevated circulating IL-10 concentrations on the host may result in the generation of a systemic inflammatory response and weight loss. Some of the apparent discrepancies in IL-10 concentrations and outcome among human cancer patients may be linked to the suggestion that IL-10 has both tumor-promoting (immunosuppressive) and tumor-inhibiting properties (inhibition of tumor angiogenesis by down-regulation of vascular endothelial growth factor) (35).

To further investigate the differential levels of cytokine expression within different body compartments, cytokine concentrations were measured in samples of tumor tissue and compared with host cytokine genotype and with rates of weight loss. Despite the link between the *IL10 GG* genotype and increased rates of weight loss in these patients, there was no association between *IL10* genotype and tumor tissue cytokine concentrations. Therefore, the *IL10* genotype was associated with rates of weight loss and with markers of systemic inflammation but not

TABLE 6
Multiple regression analysis of variables associated with increased weight loss[1]

	Hazard ratio	95% CI	*P* value
Dietary intake	3.5	1.82, 5.11	<0.001
ln C-reactive protein	0.6	−0.19, 1.41	0.134
Stage of disease	1.4	0.26, 2.58	0.017
IL10 genotype	2.0	0.52, 3.43	0.008

[1] Dietary intake, stage of disease, and *IL10* genotype retained an independent association with weight loss.

TABLE 7
Cox univariate regression analysis of variables associated with the risk of cachexia (weight loss >10%), stratified by *IL10* genotype

	Hazard ratio	95% CI	*P* value
AG vs *AA*	1.3	1.12, 1.94	0.019
GG vs *AA*	2.3	1.18, 4.30	0.014

with measurable differences in either serum or tumor tissue cytokine concentrations. In contrast, the *IL6 –174 CC* genotype was associated with significantly elevated tumor tissue cytokine concentrations. The *IL6 CC* genotype was also associated with elevated serum CRP concentrations in the current study and previously (14). IL-6 overexpression has been shown to be associated with a pancreatic cancer phenotype linked with inflammation and cachexia (15). However, the present study did not identify any association between *IL6* genotype and extent of weight loss. Therefore, both *IL10 GG* and *IL6 CC* were associated with elevated markers of systemic inflammation (elevated serum CRP concentrations), but each with differing tumor cytokine profiles and each with different relations to weight loss/cachexia. It is possible that IL-10 concentrations within other target tissues, such as muscle tissue, are more relevant to the development of cachexia.

Multivariate analysis was used to further investigate the potential association between *IL10* genotype, systemic inflammation, and increased rates of weight loss. When coanalyzed with CRP concentration, the *IL10* genotype retained an independent association with weight loss. These findings further support the potential role of IL-10 acting at a local level within target tissues to promote accelerated weight loss among these patients. This interesting and novel observation warrants further investigation in larger independent patient cohorts to confirm the present findings. Phase III studies might be designed to determine whether cytokine genotyping might be useful in stratifying patients for nutritional or pharmacologic interventions designed to prevent or intervene early in the course of cancer cachexia. Such a trial may involve genotyping patients at the time of diagnosis to identify which patients may be at increased risk of accelerated weight loss. Maximal nutritional support may then be directed to those patients most at risk of cachexia. Alternatively, novel pharmacologic interventions may be tried to counter the accelerated weight loss. Assessments could then be made to determine whether increased nutritional surveillance among this high-risk group could influence the rate of weight loss. This may then lead to directed nutritional therapy for individual patients based on their genotype risk.

Clearly, cancer cachexia is difficult to define precisely. For this reason, the definition of the cachexia phenotype adopted in the present manuscript is somewhat arbitrary and contributes to the complexity of interpreting the data.

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