

New genetic signatures associated with cancer cachexia as defined by low skeletal muscle index and weight loss

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Background Cachexia affects the majority with advanced cancer. Based on current demographic and clinical factors, it is not possible to predict who will develop cachexia or not. Such variation may, in part, be due to genotype. It has recently been proposed to extend the diagnostic criteria for cachexia to include a direct measure of low skeletal muscle index (LSMI) in addition to weight loss (WL). We aimed to explore our panel of candidate single nucleotide polymorphism (SNPs) for association with WL +/- computerized tomography-defined LSMI. We also explored whether the transcription in muscle of identified genes was altered according to such cachexia phenotype

Methods A retrospective cohort study design was used. Analysis explored associations of candidate SNPs with WL ($n = 1276$) and WL + LSMI ($n = 943$). Human muscle transcriptome ($n = 134$) was analysed using an Agilent platform.

Results Single nucleotide polymorphisms in the following genes showed association with WL alone: GCKR, LEPR, SELP, ACVR2B, TLR4, FOXO3, IGF1, CPN1, APOE, FOXO1, and GHRL. SNPs in LEPR, ACVR2B, TNF, and ACE were associated with concurrent WL + LSMI. There was concordance between muscle-specific expression for ACVR2B, FOXO1 and 3, LEPR, GCKR, and TLR4 genes and LSMI and/or WL ($P < 0.05$).

Conclusions The rs1799964 in the TNF gene and rs4291 in the ACE gene are new associations when the definition of cachexia is based on a combination of WL and LSMI. These findings focus attention on pro-inflammatory cytokines and the renin-angiotensin system as biomarkers/mediators of muscle wasting in cachexia.

Keywords Cancer; Cachexia; Polymorphisms; Genetics

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Introduction

Cachexia affects the majority of patients with advanced cancer and is associated with a reduction in treatment tolerance, response to therapy, quality of life, and duration of survival.¹ Cachexia is a complex multifactorial syndrome characterized by weight loss (WL) and specific losses of muscle and/or

adipose tissue.² Based on current knowledge, it is not possible to predict who will develop cancer cachexia and who will not. Such variation may partly be due to genotype. Knowledge of genotypic variation could contribute to early identification of risk and allow institution of prophylaxis.

Using a candidate gene approach, research by our group identified cancer cachexia with several single nucleotide

polymorphisms (SNPs); among these, a variant from the SELP gene (P-selectin³) was investigated for functional significance. Since then, many new target genes have been reported^{4–10}; these genes are involved in the key mechanisms thought to contribute to cancer cachexia, and their transcripts have been shown to play significant roles in the regulation of pathways such as muscle and adipose tissue homeostasis. We have recently published a review of candidate genes and polymorphisms in cancer cachexia.^{11,12}

Although there is depletion of both adipose tissue and lean body mass in cancer cachexia, WL *per se* has long been used as the diagnostic criterion, and this remains in current classification systems.¹³

However, skeletal muscle loss may have the greatest impact on patients' function and quality of life. It has recently been possible to quantify muscle mass in cancer patients' diagnostic computerized tomography (CT) scans, and low skeletal muscle index (LSMI) so identified is associated with poor outcome.^{13–15} One limitation is that due to the absence of pre-illness scans, it is not possible to document active muscle loss but rather LSMI determined by pre-determined cut-offs. In the present study, we use LSMI as synonymous with sarcopenia defined by cut-offs related to excess mortality.^{16,17} The combination of LSMI and WL has been suggested to combine a focus on muscle mass with a dynamic process of active loss.¹³ We used >2% WL because this is the minimal level associated with an increased risk of mortality.¹⁷ Such a combined definition proved superior to its individual components in identification of cancer patients with skeletal muscle fibre atrophy.¹⁸

We utilized a candidate gene approach to explore our hypothesis that inter-individual variations in susceptibility to cachexia are partly due to inherited genetic variations (host); remaining phenotypic variance may be ascribed to the tumour or other comorbidity. One limitation was the lack of large bio-banks characterized for cachexia phenotypes. We developed such a bio-bank with our primary objective to compare our entire panel of candidate SNPs and their association with WL with and without LSMI. We also investigated whether genes demonstrating significant associations had altered transcript expression in muscle from cancer patients with or without those phenotypes.

Materials and methods

Genotyped cancer patients: new and prior study cohorts

Subjects were recruited between 2004 and 2012 from the National Health Service Lothian, UK; Cross Cancer Institute, Edmonton, Canada; McGill University Health Centre, Montreal, Canada; Palliative Research Centre, Norwegian

University of Science and Technology, Norway; Cantonal Hospital, St Gallen, Switzerland; and Department of Medical Oncology, University Hospital of Larissa, Greece (*Table 1*). All subjects participated in clinical or research studies on cancer cachexia at the host institutions under ethically approved protocols allowing for analysis of patients' DNA. Recruitment was on presentation to surgical, oncology, or palliative care clinics. Recruitment was sequential with the following exclusions: (i) <18 years; (ii) cognitive impairment; (iii) underlying infection; and (v) on corticosteroids. Overall, 1276 patients were included (*Table 1*). More than 98% were of European descent. Information on patients included date of birth, date of diagnosis, and type and stage of cancer. Height and weight were measured upon recruitment (at time of diagnosis of cancer). Pre-morbid weight was recalled and verified where possible from the medical notes. WL was calculated and expressed as percentage of pre-morbid body weight lost. The documentation of WL depends on accurate recall. Studies in healthy populations suggest a strong correlation between recalled and measured weight.¹⁹ CT scans closest to the time of diagnosis (within 30 days on average) were selected. About 943 patients were informative for cachexia according to WL and LSMI. All patients provided written informed consent for analysis of their DNA.

Table 1 Patient demographics

	n = 1276
Age (years) ^a range	65 ± 13 (22–97)
Sex	
M	779 (61)
F	497 (39)
Tumour type	
Oesophageal or gastric	405 (32)
Pancreatic	158 (13)
Lung	550 (43)
Other	163 (12)
Stage	
I	77 (6)
II	110 (9)
III	664 (52)
IV	425 (33)
Body mass index (kg/m ²) ^a Range	25 ± 5 (13–59)
Percentage weight loss ^a	6 ± 9
Skeletal muscle index cm ² /m ^{2ab}	
M	49 ± 9
F	41 ± 7

Patients recruited from 2004 to 2012 at the NHS Lothian, UK; Cross Cancer Institute, Edmonton, Canada; McGill University Health Centre, Montreal, Canada; Palliative Research Centre, Norwegian University of Science and Technology, Norway; Cantonal Hospital, St Gallen, Switzerland; and Department of Medical Oncology, University Hospital of Larissa.

Values are number of patients with percentages in parentheses unless indicated otherwise.

^aValues are mean ± SD. Characteristics were measured at first presentation to a surgical or oncology clinic.

^bSkeletal muscle index calculated as lumbar total muscle cross-sectional area (cm²)/height (m²)

Skeletal muscle transcriptome study

Patients who contributed to the muscle transcriptomic biobank have been described recently.²⁰ Review of medical charts and CT images identified WL status and muscularity.

Phenotypes

- WL >5%, >10%, >15%. A range of WL was used to provide a subgroup analysis to identify associations that would have been missed with a single cut-off: the interest is to detect all potential associations in a polygenic model where the variants are likely to be of lower penetrance yet conferring finite effects.
- LSMI with any degree of WL (>2%): analysis of CT scans allows classification as LSMI or not. Cut-offs for LSMI were defined in relation to survival duration of advanced cancer patients.¹⁷

Computerized tomography analysis

Digitally stored CT images completed with a spiral CT were analysed as described previously. Cross-sectional area for muscle was normalized for stature (cm^2/m^2) and a lumbar skeletal muscle index (SMI) computed.^{16,21} SMI cut-offs for LSMI were based on a CT-based study of cancer patients by Martin *et al.*¹⁷

Candidate gene and single nucleotide polymorphisms selection

Candidate genes and SNP selections were based on a systematic literature review.^{11,12} Candidate SNPs met the following criteria: previously published association with cancer cachexia,^{22–24} statistically significant association with cancer cachexia in our prior study but still requiring validation,³ likely role in cancer cachexia based on functional or clinical relevance in more than one study,¹² significant SNPs identified in a preliminary study,²⁵ and those SNPs that had been identified in relation to pro-inflammatory/anti-inflammatory pathways, neuronal melanocortin signalling pathways, energy regulation, appetite regulation, muscle, and adipose tissue catabolic pathways since our prior study.¹²

Genotyping

Genotyping was performed on the Sequenom iPLEX Gold platform (San Diego, CA, USA) or TaqMan assay (for rs4280262) using services from the McGill University and Genome Quebec Innovation Centre, Montreal, Quebec, Canada. Polymorphisms selected were validated for assay feasibility

using DNA from healthy Caucasians ($n=92$) (Coriell Panel, Coriell Institute of Medicine, CA, USA).²⁶ Of the 148 SNPs selected initially (21 SNPs from a previous association study and 127 newly selected SNPs for this study), for Sequenom platform, 15 SNPs failed at the multiplex assay design stage, and 15 SNPs were non polymorphic, leaving 118 SNPs for genotyping. Assay duplicates for 154 samples genotyped for all 118 SNPs; 100% concordance for replicates was obtained. Of the 1452 patient samples, detailed clinical annotations for the study end points were available for 1276 patients (*Table 1*). Germline DNA isolated from buffy coat cells from these 1276 individuals were interrogated for the 118 SNPs. SNP call rates >90% were retained for all subsequent analysis (two SNPs did not meet this criteria; rs4280262 and rs1544410: call rates of 80 and 86%, respectively). Three SNPs showed a minor allele frequency <5%, and these were excluded (rs1805086; rs2536; and rs16139), leaving 113 SNPs from a total of 62 genes (Supporting Information *Table S1*). Deviations from Hardy–Weinberg equilibrium (HWE) were assessed in the Coriell panel of controls using the χ^2 test with 1 degree of freedom; a P -value of <0.001 was considered significant deviation from the HWE proportions. None of the 118 SNPs considered for association analysis showed deviations from HWE.

Microarray analysis

Microarray analysis was conducted as previously described.²⁰ The data used in this publication have been deposited in the US National Centre for Biotechnology Information Gene Expression Omnibus25 and are accessible through GEO series accession number GSE41726.

Power calculations

Power calculations used Quanto. For the most prevalent cachexia phenotype (i.e. >5% WL, 50% affected), the present study has 87% power to detect an odds ratio of 1.5 for SNPs with a mean allele frequency of >0.05. For the least prevalent cachexia phenotype (i.e. >15% WL, 16% affected), the present study has 35% power to detect an odds ratio of 1.5 for SNPs with a mean allele frequency of >0.05.

Statistical analysis

Gene association study

Statistical analysis was conducted as previously described.³ Briefly, analyses were performed using PLINK (version 1.06).²⁷ Analyses were adjusted for covariates: age at diagnosis, sex, pre-diagnosis body mass index, tumour type, and stage. Patients meeting the criteria for each of the cachexia phenotypes were compared with patients who had lost

<5% body weight as control. To account for multiple testing, permutation testing was performed using the adaptive permutation test in PLINK within each phenotype. Finally, candidate genes (and the SNPs in the corresponding gene regions) were grouped on functional similarity according to gene ontology (AmiGO) (Supporting Information Table S2). The set-based test in PLINK was used to analyse association between grouped SNPs and cachexia = phenotypes. The latter selects the best set of SNPs whose mean of these single SNP statistics is significant after permutation, which is particularly suited to large-scale candidate gene studies.²⁸ The empirical *P*-values were obtained by a permutation of 10 000 times of phenotype labels.

Transcriptomic study

Pearson correlation analysis assessed the relationship between the phenotypes independently (SMI or WL) with the expression of transcripts from select candidate genes. *t*-test compared how SMI or WL values differed with high vs. low expression for each of the candidate genes. The high and low groups were determined by expression intensity and splitting patients into three equal groups. The extremes were compared while leaving out middle values. Cases considered for SMI and WL phenotypes for gene expression were based on sorting of transcript expression in all samples and binning based on extremes as described earlier. The samples used for SNP studies and gene expression studies are from non-matched cases as these two were independent studies.

Results

Characteristics of the patient population are presented in Table 1. Average age was 65 ± 13 years (mean ± SD). The majority was stage III or IV. Average WL was 6 ± 9%. Of the patients with CT scans for the assessment of muscularity, 47% had LSMI. There were no significant differences in age, stage of disease, pre-diagnosis body mass index, and percentage WL between patients who had CT scans suitable for the measurement of muscularity and the entire cohort (Table 1).

Weight loss alone phenotype (n = 1276)

Table 2 lists results for SNPs associated with cancer cachexia in patients classified according to WL alone. Sixteen SNPs had significant associations with various cachexia phenotypes based on increasing severity of WL. Two SNPs (rs1935949 and rs4946935) found within chromosome 6 in the Forkhead box O3 (FOXO3) gene associated with WL of increasing severity (>5% and >10%) and one SNP (rs2297627) found in the Forkhead box O1 (FOXO1) gene associated with WL > 10%.

Table 2 Genes with variants significantly associated with cancer cachexia in patients classified according to weight loss alone

% Weight loss	Gene	SNP	Risk allele	OR (95%CI)	Permutated <i>p</i>
5	GCKR	rs1647266	C	0.786 (0.664–0.931)	0.006
5	LEPR	rs1137100	G	0.781 (0.647–0.942)	0.012
5	GCKR	rs780106	C	0.802 (0.678–0.949)	0.012
5	SELP	rs6136	C	0.677 (0.504–0.908)	0.013
5	ACVR2B	rs2268757	C	1.219 (1.032–1.440)	0.035
5	TLR4	rs1554973	C	1.237 (1.013–1.510)	0.038
5	FOXO3	rs1935949	T	1.241 (1.033–1.491)	0.039
5	FOXO3	rs4946935	A	1.224 (1.019–1.470)	0.042
10	LEPR	rs1137100	G	0.665 (0.524–0.843)	0.001
10	SELP	rs6136	C	0.514 (0.345–0.766)	0.001
10	IGF1	rs35767	T	0.681 (0.510–0.910)	0.012
10	FOXO3	rs1935949	T	1.306 (1.047–1.630)	0.013
10	CPN1	rs11597390	A	1.237 (1.007–1.519)	0.027
10	LEPR	rs12409877	A	0.793 (0.639–0.984)	0.033
10	FOXO3	rs4946935	A	1.277 (1.023–1.594)	0.035
10	APOE	rs157580	G	1.239 (1.005–1.528)	0.047
10	FOXO1	rs2297627	C	0.793 (0.637–0.988)	0.049
15	SELP	rs6136	C	0.433 (0.247–0.757)	0.005
15	LEPR	rs5010905	C	1.551 (1.138–2.112)	0.007
15	IGF1	rs35767	T	0.626 (0.430–0.912)	0.010
15	LEPR	rs1137100	G	0.665 (0.486–0.909)	0.011
15	CPN1	rs11597390	A	1.312 (1.012–1.701)	0.040
15	CPN1	rs1049353	A	0.711 (0.514–0.984)	0.042
15	GHRL	rs42451	T	1.344 (1.012–1.785)	0.047

CPN1, Carboxypeptidase N polypeptide 1; FO, Forkhead box; LEPR, leptin receptor; OR, odds ratio; SELP, P-selectin; SNP, single nucleotide polymorphism.

Weight loss >5%, number affected: 633/1276 (49.6%); weight loss >10%, number affected: 382/1276 (29.9%); weight loss >15%, number affected: 199/1276 (15.6%).

Weight loss plus low skeletal muscle index phenotype (n = 943)

Table 3 lists all SNPs associated significantly with cancer cachexia classified according to LSMI + WL >2% in all recruited patients. The analysis compared those with the LSMI + WL >2% phenotype against those without in the entire cohort. rs12409877 is in the leptin receptor (LEPR) located on chromosome 3. rs2268757 is located in the activin receptor type-2B (ACVR2B) gene on chromosome 3. SNPs in the tumour necrosis factor (TNF) (rs1799964) and ACE (rs4291) genes also associated with the phenotype.

Combining genes with functional similarity according to gene ontology

Table 4 lists the phenotypes for candidate gene groups associated with specific cancer cachexia phenotypes. SNPs in groups of genes involved in appetite regulation, cell adhesion, cell membrane structure and function, and signal transduction were associated with the phenotype WL >10%. Only SNPs in the group of genes involved in cell

Table 3 Genes with variants significantly associated with cancer cachexia in patients classified according to weight loss >2% and low skeletal muscle index compared with those who do not

Gene	SNP	Risk allele	OR (95%CI)	Permutated <i>p</i>
LEPR	rs12409877	A	0.674 (0.526–0.865)	0.002
ACVR2B	rs2268757	C	1.406 (1.126–1.757)	0.002
TNF	rs1799964	C	1.435 (1.093–1.885)	0.010
ACE	rs4291	T	1.313 (1.039–1.659)	0.025

LEPR, leptin receptor; OR, odds ratio; SNP, single nucleotide polymorphism; TNF, tumour necrosis factor.

WL >2% + LSMI. Number affected: 214/943 (22.7%).

Table 4 Candidate gene groups associated with cancer cachexia phenotypes

Phenotype	Candidate gene group function	Number of genes ^a	Number of SNPs	<i>P</i> -values
Weight loss >5%	N/A	N/A	N/A	N/S
Weight loss >10%	Appetite regulation	8	21	0.004
	Cell adhesion	12	17	0.005
	Cell membrane structure and function	32	66	0.037
Weight loss >15%	Signal transduction	51	110	0.038
	Cell adhesion	12	17	0.019
LSMI + weight loss >2%	Appetite regulation	8	21	0.014
	Signal transduction	51	110	0.023
	Glucocorticoid signalling	4	6	0.034
	Lipid metabolism	15	38	0.039

LSMI, low skeletal muscle index; N/A, not applicable; N/S, not significant; SNP, single nucleotide polymorphism.

^aThe genes in each candidate gene group are listed in Supporting Information Table S2

adhesion were significant with increasing WL. SNPs in groups of genes involved in lipid metabolism, appetite regulation, signal transduction, and glucocorticoid signalling were associated with the phenotype LSMI and WL >2%. No SNPs in groups of genes were found to be significant with all other phenotypes.

Transcriptomic analysis

Table 5 lists the results from correlation and *t*-test analysis between phenotypes and gene transcript level for the genes that showed significant associations with any of the cachexia phenotypes. Expression of ACVR2B, FOXO1 and 3, GCKR, LEPR, and TLR4 transcripts was significantly associated with different levels of SMI or WL ($P < 0.05$). Specifically, these were all negatively correlated with muscularity. FOXO1 and 3 and GCKR were the only genes significantly correlated with WL; these were correlated negatively with WL.

Discussion

Associations with different cachexia phenotypes

In the present study, four SNPs are associated with WL + LSMI (Table 3). Two of these SNPs are associated with muscle metabolism in two genes (ACVR2B and ACE), one with fat metabolism in one gene (LEPR) and one with cytokine production in one gene (TNF). It would be attractive to assign specific functional significance to the genetic signatures identified. For example, ACVR2B decoy receptors abrogate muscle loss and prolong survival in several murine models of cancer cachexia.⁷ rs1799964 in the TNF gene and rs4291 in the ACE gene are new associations (c.f. WL alone) when classification is based on WL + LSMI. These findings focus attention on pro-inflammatory cytokines and the renin–angiotensin system as biomarkers/mediators of muscle wasting in cachexia. Replication of the present findings along with genome-wide scans and an imputation approach to fine map the loci are needed in parallel with functional studies (see the following) to resolve this issue further.

For the WL phenotype, sixteen candidate SNPs were identified (Table 2). Seven of these SNPs are associated with muscle metabolism in five genes (IGF1, CPN1, FOXO1, FOXO3, and ACVR2B), four are associated with adipose tissue metabolism in two genes (LEPR and APOE), two with the immune response in two genes (SELP and TLR4), two with corticosteroid signalling in one gene (GCKR), and one with appetite regulation in one gene (GHRL). Two polymorphisms (rs1935949 and rs4946935) in the gene encoding for FOXO3 were consistently associated with WL of increasing severity (>5% and >10%) (Table 2). On the basis that WL is a continuum, the observation that both SELP and FOXO3 associate with the highest degrees of WL suggests that these signatures may be of particular significance. A recent study in a mouse model of cancer cachexia demonstrated that FOXO-dependent transcription is key in controlling diverse gene networks in skeletal muscle during cancer cachexia.²⁹

In keeping with our prior study,³ we confirmed in a larger validation cohort (Stage 2, $n = 545$) that patients who carry the C allele of the rs6136 SNP in the SELP gene are at a reduced risk of cachexia defined by WL (>5%, >10%). This was confirmed recently in chemo-naïve patients with locally advanced or metastatic pancreatic cancer.³⁰

Gene group analysis

The two dominant mechanisms of WL in cancer are anorexia/reduced food intake and abnormal metabolism.¹³ Appetite regulation was found to associate with the cachexia trait WL >10% ($P = 0.0041$). Regarding metabolism, lipid metabolism associated with LSMI and WL >2% ($P = 0.0138$). Fatty infiltration (myosteatosis) has been associated with cancer

Table 5 Results from correlation and *t*-test analysis between patient characteristics and rectus abdominus muscle gene transcripts for selected genes^a

Probe name	Gene symbol	Correlation between probes within genes ^b	Correlation with SMI, cm ² /m ² (<i>n</i> = 102) ^c	Correlation with % weight loss, %/100d (<i>n</i> = 86) ^c	FD ^d	<i>t</i> -test of SMI values for patients with high vs. low probe expression <i>P</i> -value ^e	<i>t</i> -test of % weight loss/100d values for patients with high vs. low probe expression <i>P</i> -value ^e
A_23_P109950	ACVR2B	0.94	-0.21	-0.03	3.9	0.04	0.86
A_24_P231132	ACVR2B		-0.24	-0.09	3.0	0.03	0.17
A_23_P151426	FOXO1	0.71	-0.22	-0.23	4.2	0.01	0.04
A_24_P22079	FOXO1		-0.39	-0.43	3.5	< 0.01	< 0.01
A_23_P345575	FOXO3	0.97	-0.31	-0.29	2.9	< 0.01	0.01
A_32_P102062	FOXO3		-0.33	-0.28	3.1	< 0.01	0.01
A_23_P119886	GCKR		-0.03	0.06	3.8	0.80	0.03
A_23_P161135	LEPR		-0.26	0.00	3.5	< 0.01	0.31
A_23_P60306	TLR4	0.60 to 0.85	-0.18	-0.07	2.1	0.02	0.19
A_24_P69538	TLR4		-0.10	-0.01	2.1	0.03	0.95
A_32_P66881	TLR4		-0.19	-0.15	2.0	0.04	0.29

ACE, angiotensin converting enzyme; ACVR2B; activin receptor type-2B; CPN1, carboxypeptidase N polypeptide 1; FD, fold difference; FOX, forkhead box; IGF1, insulin-like growth factor 1; LEPR, leptin receptor; SMI, skeletal muscle index; TNF, tumour necrosis factor.

^aThe following gene probes did not show significant Pearson correlation or significance for the *t*-test analysis: ACE probes (A_23_P371777, A_23_P38235, A_24_P365129), ACVR2B probe (A_32_P134209), APOE probe (A_23_P164650), CNR1 probes (A_23_P214208, A_24_P363259), CPN1 probe (A_23_P98147), GHRL probe (A_23_P40956), IGF1 probes (A_23_P13907, A_24_P304419, A_24_P304423, A_24_P398572), LEPR probe (A_24_P231104), SELP probe (A_23_P137697), TNF probes (A_23_P376488, A_24_P50759).

^bPearson correlation analysis was conducted only for genes with multiple probes.

^cPearson correlation analyses were conducted to identify linear relationships between gene probe intensities and SMI or weight loss. Note that not all 134 patients had both SMI and weight loss information available, and therefore, the number of patients for the SMI and weight loss correlation analysis were 102 and 86, respectively.

^dFold change = average high expressors/average low expressors.

^eThe average sample size for the *t*-test comparing the SMI values for patients with high vs. low probe expression was 35 and 33, respectively. The average sample size for the *t*-test comparing the % weight loss/100d values for patients with high vs. low probe expression was 30 and 29, respectively. These sample sizes differed slightly from test to test as not all patients had computing tomography scans for SMI measurements and not all patients had weight loss values in their clinical charts.

Pearson correlation *P*-value < 0.05.

Bold figures are the ones that are significant.

cachexia and reduced survival.^{17,31} The glucocorticoid signalling pathway also associate with LSMI and WL >2% (*P*=0.0337). Glucocorticoids and associated signalling pathways accelerate protein degradation in muscle.³²

The muscle transcriptome is altered in the presence of cancer cachexia.^{33,34} In the present study, there was concordance between a proportion of the selected genes and either the level of WL or muscularity (Table 5). FOXO1 and FOXO3 are good examples: SNPs in both genes associated with the WL phenotype (Table 2) and transcript levels of both showed a correlation with WL (Table 5). These transcription factors are not only key in the pro-inflammatory driven up-regulation of the ubiquitin-proteasome pathway but also act as negative regulators of the anabolic Akt-mTOR pathway.^{8,35}

The present SNP analysis was not genome-wide, and therefore, other variants with possible functional significance may have not have been examined. Equally, the true functional significance of any individual SNP is mostly unknown. It may be better to consider the genetic associations identified as genetic signatures or biomarkers associated with the cachexia syndrome. Interestingly, 17 of the 19 SNPs reported as showing significant associations are in intronic, 3', or 5' Un translated regions (UTRs). The

purpose was to probe into the potential functional impact of the loci as SNPs in this study are potentially proxy to the causal variants (not yet captured in the region), which may also have an influence on gene expression; as such, the probe position in the expression array and the SNP position are not the same. Extrapolation to an SNP under being an expression quantitative trait loci is premature. The SNP identified may in some cases also affect gene expression signatures not addressed herein (as in cis-acting and trans-acting expression quantitative trait loci). The correlation pattern (albeit, low to modest) observed is still encouraging because the trends reported here for an SNP loci and gene expression are within the scope of known cachexia literature. There is also a growing body of evidence that microRNAs are involved in cancer cachexia,³⁶ and it may be that the newly discovered SNPs alter the gene transcripts of these highlighted genes. Animal models may well be useful to look at the biology of altering the transcripts from the genes where the SNPs were found.

Equally, for those genes for which no strong relationship was found between gene expression and patient characteristics, it is important to consider that these may not be transcriptionally regulated. For systemic mediators (e.g. cytokines), it may be that circulating concentration is

important rather than local expression because tissue-specific expression may be transient, but the activation of the signal transduction cascade could be the largest prevailing effect.

It is important that the prevalence of LSMI is in excess of that observed in the normal age-matched population. The prevalence of LSMI/sarcopenia in age-matched subjects living in the community varies according to the definition and methodology used but is reported between 1 and 29%.³⁷ The prevalence of LSMI in this study was ~48%. Thus, the gene associations with LSMI represent associations with a level of muscularity at least partly independent of age, sex, or stature. Clearly, there are other reasons why cancer patients may lose muscle mass and weight apart from their tumour-related cachexia, e.g. severe chronic obstructive pulmonary disease. Such co-morbidities were not graded prospectively in the current study but should be considered for the characterization of future cohorts.

Conclusions

Candidate gene SNP analysis offers the advantage that it is hypothesis-driven and the associations are easily explained owing to compelling biological rationale. However, the limitations are that the role of hitherto unexplored genes and pathways that otherwise contribute to the trait under investigation are missed. Issues surrounding phenotype complexity are addressed in part in this study, and conducting a genome-wide association study using high density of markers on the genome would help relate the overlap of SNPs/pathways to the phenotypes of interest. The consensus definitions for phenotypes may evolve in an iterative manner from the cumulative wisdom from candidate SNPs, genome-wide association study, and the current definitions available for cachexia. This could potentially lead to the discovery of new SNPs depending on the phenotype chosen.

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Conflict of interest

No authors declare a conflict of interest.

Authors' contributions

N.J., B.T., J.R., V.B., S.D., and K.F. designed research.

N.J., C.S., and S.D. conducted research.

N.J., C.S., B.T., T.S., S.S., N.S., I.G., R.S., C.D., A.V., O.B., M.T., S.K., F.S., B.G., and V.B. provided essential reagents or provided essential materials.

N.J. and C.S. analysed data and performed statistical analysis.

N.J., C.S., V.B., S.D., and K.F. wrote the paper.

N.J., V.B., S.D., and K.F. had primary responsibility for final content.

Abbreviations

ACE: (angiotensin converting enzyme)

ACVR2B: (activin receptor type-2B)

AKT: (protein kinase B)

BMI: (body mass index)

CAM: (cell adhesion molecule)

COPD: (Chronic obstructive pulmonary disease)

CPN1: (carboxypeptidase N polypeptide 1)

CT: (computerized tomography)

DNA: (deoxyribonucleic acid)

eQTL: (expression quantitative trait loci)

FOX: (Forkhead box)

IGF1: (insulin-like growth factor 1)

LEPR: (leptin receptor)

LSMI: (low skeletal muscle index)

LPIN2: (LIPIN 2)

MAF: (mean allele frequency)

MT2A: (metallothionein 2A)

mTOR: (mammalian target of rapamycin)

NCBI: (National Centre for Biotechnology Information)

NF- κ B: (nuclear factor kappa-light-chain-enhancer of activated B cells)

NTNU: (Norwegian University of Science and Technology)

OR: (odds ratio)

PPARG: (peroxisome proliferator-activated receptor gamma)

RIN: (RNA integrity number)

RNA: (ribonucleic acid)

SD: (standard deviation)

SELP: (P-selectin)

SMI: (skeletal muscle index)

SNP: (single nucleotide polymorphism)

TLR4: (toll like receptor 4)

TNFRSF1A: (tumour necrosis factor receptor superfamily member 1A)

UTR: (Un-Translated Region)

WDR20: (WD Repeat Domain 20)

χ^2 : (chi-squared)

Online supplementary material

Supporting information may be found in the online version of this article.

Supplementary Table 1 List of polymorphisms considered for association analysis

Supplementary Table 2 Candidate genes groupings based on known functional similarity according to gene ontology

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