REVIEW ARTICLE

Genetic basis of interindividual susceptibility to cancer cachexia: selection of potential candidate gene polymorphisms for association studies

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Abstract

Cancer cachexia is a complex and multifactorial disease. Evolving definitions highlight the fact that a diverse range of biological processes contribute to cancer cachexia. Part of the variation in who will and who will not develop cancer cachexia may be genetically determined. As new definitions, classifications and biological targets continue to evolve, there is a need for reappraisal of the literature for future candidate association studies. This review summarizes genes identified or implicated as well as putative candidate genes contributing to cachexia, identified through diverse technology platforms and model systems to further guide association studies. A systematic search covering 1986–2012 was performed for potential candidate genes / genetic polymorphisms relating to cancer cachexia. All candidate genes were reviewed for functional polymorphisms or clinically significant polymorphisms associated with cachexia using the OMIM and GeneRIF databases. Pathway analysis software was used to reveal possible network associations between genes. Functionality of SNPs/genes was explored based on published literature, algorithms for detecting putative deleterious SNPs and interrogating the database for expression of quantitative trait loci (eQTLs). A total of 154 genes associated with cancer cachexia were identified and explored for functional polymorphisms. Of these 154 genes, 119 had a combined total of 281 polymorphisms with functional and/or clinical significance in terms of cachexia associated with them. Of these, 80 polymorphisms (in 51 genes) were replicated in more than one study with 24 polymorphisms found to influence two or more hallmarks of cachexia (i.e., inflammation, loss of fat mass and/or lean mass and reduced survival). Selection of candidate genes and polymorphisms is a key element of multigene study design. The present study provides a contemporary basis to select genes and/or polymorphisms for further association studies in cancer cachexia, and to develop their potential as susceptibility biomarkers of cachexia.

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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. Cancer cachexia has recently been defined as a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment (Fearon *et al.* 2011). Depending on the tumour type, weight loss occurs in 30–80% of cancer patients and is severe (with loss of >10% of the initial body weight) in 15% (Dewys *et al.* 1980). The degree of cancer cachexia is variable depending on the phenotype and genotype of both patient and tumour. It is likely, there may be cachexia prone genotypes as well as cachexia resistant genotypes. A recent longitudinal study of patients with a variety of cancers has demonstrated that some will remain

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stable, lose or gain skeletal muscle or adipose tissue (Prado *et al.* 2013), further strengthening the concept of a genetic predisposition to wasting in the presence of cancer.

Pathophysiology of cancer cachexia is characterized by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism (Fearon *et al.* 2011). Skeletal muscle loss appears to be the most significant event in cachexia leading to poor treatment outcomes (Tan *et al.* 2009; Fearon *et al.* 2011). While malnutrition is reversible with nutrient intake, cachexia is not completely reversible by this approach. Indeed, cachectic patients usually present with progressive weight loss along with body composition alterations and disturbed homeostasis of many body systems, particularly of fat tissue and muscle (Tisdale 2002; von Haehling *et al.* 2009).

Single nucleotide polymorphisms (SNPs) are the most common type of heritable and evolutionarily stable genetic variations in the population (Brookes 1999); other genetic polymorphisms include copy number aberrations, insertion, deletions and tandem repeats. SNPs may exert differing effects on genes leading to an aberrant gene product. Polymorphisms in promoter regions potentially contribute to differential gene expression, presumably affecting the binding of transcription factors to DNA. Sequence variation in the 5′ untranslated region (UTR) could disrupt mRNA translation; mutations in the 3′ UTR could affect mRNA through posttranscriptional mechanisms such as splicing, maturation, stability and export. Polymorphisms in intronic regions may result in *cis* regulation or *trans* regulation of genes, unmask cryptic splice sites or promoters leading to alternative transcripts. Synonymous and nonsynonymous SNPs in exons could alter protein function or activity and may introduce codon bias contributing to the relative abundance of the proteins, respectively. Finally, nonsense mutations cause a stop altogether in the translation of mRNA (Wjst 2004). Genomic distribution of SNPs is not homogenous, SNPs usually occur in noncoding regions more frequently than in coding regions (Barreiro *et al.* 2008). It has been estimated that 10% of all SNPs in the genome are functional, thereby having the potential of altering some biological process.

The case to support a genetic predisposition to cachexia is strengthened from the known genetic contribution to the activity of a variety of key mechanisms that underlie the cachexia syndrome. In a previous systematic review on the identification of possible genetic polymorphisms involved in cancer cachexia, a total of 184 polymorphisms with functional or clinical relevance to cancer cachexia were identified in 92 candidate genes (Tan *et al.* 2011). Following this approach here, we were able to identify 23 significant SNPs associated with cachexia based on definitions of weight loss and systemic inflammation (measured with C-reactive protein (CRP)) and validated a SNP in the *SELP* gene encoding for p-selectin in an independent cohort. P-selectin binds to leucocytes and in certain inflammatory conditions, the plasma concentration of soluble p-selectin is highly elevated (Dunlop *et al.* 1992). By incorporating a definition based on systemic inflammation, we identified a SNP involved in the innate immune response. To date most of the studies on identification of SNPs involved in cancer cachexia has led to the discovery of other SNPs involved in the innate immune system, mainly in the interleukin family of cytokines (Jatoi *et al.* 2007, 2010; Knoll *et al.* 2008; Zhang *et al.* 2008; Deans *et al.* 2009; Bo *et al.* 2010; Rausch *et al.* 2010; Sun *et al.* 2010a, b) (table 1). SNPs in other biological processes have also been studied for their association with cancer cachexia (Solheim 2011, 2012; Punzi *et al.* 2012) (table 1). By altering the phenotype for a degree of skeletal muscle quantification, it may be possible to identify SNPs associated with muscle tissue biology.

A contemporary review of the literature is now needed as a greater understanding of the processes involved in cancer cachexia are increasingly sought. To enable further candidate gene selection studies with larger cohorts, new targets need to be identified to maximise the potential for associations. An increase in earlier sample size will increase the power of further studies to identify novel SNPs. To accommodate the evolving phenotype definitions and the current state of the understanding of cachexia, we searched for candidate genes or pathways related to the biology of muscle, inflammation, adipose tissue, obesity, diabetes and molecular mechanisms of cancer in general, as well as factors affecting survival and prediction of outcomes following treatments. There is an express need to restrategize the selection of candidate genes to drive association studies since 'omics' approaches uncovered the hitherto unexplored biological pathways in the last five years in the realm of cancer and genetic predisposition markers for a host of diseases and traits. In view of the increased understanding of the biology of cancer cachexia, we have amended the search terms used as both biology and classification of cancer cachexia continues to evolve. We reasoned that insights into functional significance of the candidate gene SNPs will help explore putative causality of the SNPs. These approaches would likely strengthen the premise for hypothesis driven phenotype–genotype association studies for polygenic diseases/traits, *vis-à-vis* potential to identify variants with higher effect size and hence heritable component of the cachexia risk in individuals. Functionality of SNPs/genes was explored based on published literature, algorithms for detecting putative deleterious SNPs and databases for expression quantitative trait loci (eQTLs) (Hunter and Crawford 2008; Schadt *et al.* 2008; Fehrmann *et al.* 2011; Hao *et al.* 2012; He *et al.* 2013). Interindividual variations in susceptibility to cancer induced cachexia and the heritable component of the genome when fully delineated would help identify possible interventions for those at risk well before the onset of clinical symptoms.

Methods

The US National Library of Medicine database, Medline; the Excerpta Medica database, Embase; Cochrane Central Register of Controlled Trials, Central; and the database of the

Table 1. Published associations of genetic variants in cancer cachexia.

Reference	SNP	Gene	Patients included	Phenotype
Solheim (2012)	No associations	N/A	Patients 1853, with cancer at different sites, stages and with different performance status	EORTC QLQ-C30 questionnaire, question 13: 'have you lacked appetite'
Punzi (2012)	rs1544410 (*Bsm*I) rs731236 (*Taq*I)	*VDR*	Patients 43, with cancer from various sites and stages	Guidelines for diagnosis of cancer associated cachexia provided by the Italian Association of Medical Oncology
Tan (2012)	rs6136	*SELP*	Patients 775, with upper gastrointestinal cancers (UGI) and pancreatic cancers	Six phenotypes (1) >5% weight loss (2) >10% weight loss (3) >15% weight loss (4 – 6) The above with CRP concentration of >10 mg/l $^{-1}$
Solheim (2011)	No associations	N/A	Patients 1797, with cancer at different sites, stages and with different performance status	• BMI: <20 kgm^{-2} • Karnofsky score: <80 • CRP: <10 mg l^{-1} • Appetite loss: a response of little or greater on EORTC QLQ-C30 item 'have you lacked appetite?' >3 features = severe cachexia 2 or 3 features = mild cachexia <2 = no cachexia
Sun *et al.* (2010a, b)	rs1800896	*IL-10*	Two hundred and twenty-three gastric cancer	
Bo *et al.* (2010)	rs2227306	*IL-8* + 781	Gastric cancer	
Jatoi *et al.* (2010)	rs1800629	*TNF*	Patients 471, with non small cell lung cancer	>10% Weight loss
Rausch *et al.* (2010)	rs3024498	*IL-10*	Caucasian lung cancer survivors 1149	Lung cancer symptom scale How much loss appetite are you experiencing?
Deans *et al.* (2009)	rs1800896	*IL-10* (-1082)	Patients 203, with UGI cancers	>10% Weight loss
Knoll *et al.* (2008)	rs2229616 Val103Ile	*MC4R*	Patients 509, with various cancers (including haematological malignancies) at various stages	(1) >10% weight loss (exclusively cancer) (2) >10% weight loss (treatment influenced) (3) >5% weight loss (4) >1% weight loss (cancer specific) or >5% treatment induced (5) No weight loss
Zhang *et al.* (2007)	rs1143634	*IL-1*β (+3954)	Patients 214, with locally advanced gastric cancer	>10% Weight loss
Jatoi *et al.* (2007)	rs1143634	*IL-1*β (+3954)	Patients 44, with metastatic gastric and gastrooesophagal cancer	Phenotype is greater improvement in weight registered once in every three weeks during chemotherapy

BMI, body mass index.

Cumulative Index to Nursing and Allied Health Literature, Cinahl, were searched through the National Library of Health website, the Cochrane library, Pubmed (free citation database of Medline) and Grey literature online.

The first aim was to identify articles with new potential candidate genes involved in the development of cancer cachexia using the keyword search 'genes/genetics and inflammation or cancer or cachexia or weight loss or body composition or survival or muscle or adipose to provide an update on the existing database. Following the initial retrieval of possible new candidate genes, selected genes were entered into a pathway functional analysis software (ingenuity pathways analysis (IPA), ingenuity systems, Redwood, USA) to further identify related genes.

The second and third aims were to find genetic polymorphisms with known functional or clinical significance in each of these new and existing genes using the keyword search (gene of interest) and polymorphism(s). All identified candidate genes were reviewed for functional polymorphisms or clinically significant polymorphisms in terms of cachexia using OMIM, dbSNP and GeneRIF databases. The search was limited to reports within English language and a manual search of reference lists and conference proceedings followed with all cross references screened.

Study selection

Published papers and or abstracts were screened after removing duplicates. Inclusion criteria were set as any study either involving genes in the selected domains or any study showing functional SNPs influencing these genes. Reasons for exclusion included studies of irrelevance to cancer cachexia, studies with no genes or functional polymorphisms identified, and complex polygenic phenotypes analysing random SNPs for association. The remaining papers were reviewed in full and only those involving genes in the development of cancer cachexia or any study showing functional SNPs influencing these genes, were included in the final analysis.

In accordance with the previous review, candidate genes were grouped based on the role their product is postulated to have in the development of cancer cachexia (Tan *et al*. 2011), these being the domains of:

(i) Inflammation:
(a) innate immune receptors and mediators of the immune response; (b) cytokines; (c) cytokine receptors and related binding proteins; (d) acute phase protein reactants.
(ii) Central homeostasis
(a) energy production; (b) insulin-like growth factors and related proteins; (c) corticosteroid signalling proteins.
(iii) Muscle:
(a) muscle function and structure; (b) muscle synthesis; (c) muscle proteolysis and degradation.
(iv) Adipose tissue:
(a) adipogenesis; (b) lipid turnover and transport; (c) adipokines and adipokine receptors.
(v) Appetite.
(vi) Others.

The updated summary tables of polymorphisms are presented according to each category with 'easy to see' boxes that denote whether a polymorphism has any effect on inflammation, weight/body composition (i.e. lean mass / fat mass) and cancer survival. Also added is information on ancestral allele, SNP allele, type of SNP, polymorphism reference number (rs number), and mean allele frequency (MAF) based on a population with European ancestry derived from the HapMap or dbSNP databases.

Pathway analysis

Multiple genes within a single pathway are likely to influence the development of cancer cachexia. To provide a more comprehensive assessment in terms of pathway involvement in cancer cachexia, we performed pathway-based analyses using the IPA software. Focus genes were defined as functional SNPs which had been validated in at least one study and these were entered into the IPA analysis tool. The IPA software was used to measure associations of these genes with other molecules, their network interactions, and biological functions stored in its knowledge base. Our focus genes served as seeds for the IPA algorithm, which recognizes functional networks by identifying interconnected molecules, including molecules not among the focus genes from the IPA knowledge base. The software illustrates networks graphically and calculates a score for each network, which represents the approximate 'fit' between the eligible focus molecules and each network. The network score is based on the hypergeometric distribution and is reported as the −log (Fisher's exact test result).

Putative functions

To identify the likely effect of individual SNPs on nearby genes the sorting intolerant from tolerant (SIFT; http://sift.bii.a-star.edu.sg/) program was used for nonsynonymous SNPs (Ng and Henikoff 2003), SIFT is a program that predicts whether a nonsynonymous SNP leading to an amino acid substitution affects protein function or not (Ng and Henikoff 2003). To predict whether an amino acid substitution in a protein will affect protein function, SIFT considers the position at which the change occurred and the type of amino acid change. Given a protein sequence, SIFT chooses related evolutionarily conserved proteins and amino acid residues likely critical for structure or function and obtains an alignment of these proteins with the query. Based on the amino acids appearing at each position in the alignment, SIFT calculates the probability that an amino acid at a position is tolerated conditional on the most frequent amino acid being tolerated. If this normalized value is less than a cut-off, the substitution is predicted to be deleterious (Ng and Henikoff 2001). Many of the amino acid substitutions are well tolerated since the SNPs tend to capture subtle effects, and the ones with drastically altered functions are not to the survival advantage of the species, hence are eliminated during the course of evolution. A limitation of SIFT program is that only nonsynonymous substitutions are interrogated and those in the regulatory and intronic regions (>95% of the genome) are not considered.

The SNP and copy number annotation (SCAN) database (http://www.scandb.org/newinterface/about.html) is a large-scale database providing a web interface for easy search with a gene name or an input of SNP ID (rs#) (Gamazon *et al*. 2010). This database can use SNPs from 5′ or 3′ and intronic SNPs and their potential influence on the gene expression for nearby genes; gene expression if regulated on the same chromosome and within 100 Mb distance from the SNP could be categorized as *cis* regulatory SNPs, if not they are called *trans* SNPs. The SNPs are classified according to their effects on expression levels, i.e. eQTLs using scan database. Gene expression data and polymorphism data from HapMap population-derived lymphoblastoid cell lines (Caucasian, Yoruba and Han Chinese/Japanese) are used to interrogate the *cis* effects and *trans* effects from SNPs/genes. We have therefore summarized our findings for the selected SNPs for possible functional significance using this approach as well. Generalizability of observations

from genotype to expression from lymphoblastoid cell lines is not the same as correlations made with tissue specific expression, and therefore, one should exercise caution in the interpretations of findings. However, several published studies attest to the utility of this approach to extrapolating genotype–phenotype correlations to be valid even to specific tissues, albeit for select candidate SNPs.

Results

A total of 281 polymorphisms in 154 genes were identified and explored for relevance to cancer cachexia (see tables 1–15 in electronic supplementary material at http://www/ias.ac.in/jgenet/). Of these genes, 88 were newly identified and 66 were from the previous review by our group (Tan *et al*. 2011). The 281 functional polymorphisms with or without clinical significance to cancer cachexia (i.e. inflammation, loss of fat mass and/or lean mass and reduced survival) were found in 119 genes with no SNPs of interest found in the remaining 35 genes. A pathway-based analysis for relevance to cachexia and SNPs selected from these pathways are summarized (table 3; figures 1 & 2).

Inflammation

Inflammation is widely accepted to play a dominant role in the host's response to cancer and mediators regulating skeletal muscle atrophy in cachexia are thought to derive from immune or tumour cells, or the targeted tissues undergoing wasting (both adipose tissue and skeletal muscle) (Stewart *et al.* 2006). Cancer cells rely on production of proinflammatory mediators for growth, protection from apoptosis, and promotion of angiogenesis/metastasis. The tumour may consequently initiate a cytokine cascade that has multiple, direct, and distant effects including the initiation of skeletal muscle protein degradation. Host immunological response to proinflammatory and antiinflammatory cytokines dictates to what degree the metabolic rates are altered. In experimental models, proinflammatory cytokines lead to an acute phase response and tissue catabolism (Argiles *et al.* 2003).

Innate immune receptors and mediators of immune response: Electronic supplementary table 1 shows the genes involved in the regulation of the innate immune response and how they may generate or suppress the inflammatory response to influence the rate of cancer cachexia. Variants of these genes which may influence their function are also listed. Since the previous review, focus has spread to include new genes (*AKT2*, *AKT3* and *COX2*) (Bonetto *et al.* 2011; Ol *et al.* 2011) which act via signalling pathways to alter the innate immune response. AKT regulates cellular survival and metabolism by binding and regulating many downstream effectors, e.g. NF-κB and Bcl-2 family proteins (Song *et al.* 2005). IGF-1-induced AKT signalling is important in both the suppression of degradation and induction of protein synthesis (Rommel *et al*. 2001). AKT2 has been shown to induce glucose transport, a mouse model null for AKT2 demonstrated marked growth deficiency and displayed a diabetic phenotype (Garofalo *et al.* 2003); the role of AKT3 is less clear.

Also of interest are variants coding for *COX2*, it is found in low levels in most cells under normal conditions but has been shown to be elevated during periods of inflammation. *COX2* mediates the formation of prostaglandins from arachidonate and may play a role as a major mediator of inflammation and/or a role for prostanoid signalling (Kim *et al.* 2005).

New variants in the genes coding for the toll-like receptor (TLR) family and CAMs have been found. These genes play an instructive role in innate immune responses as well as the subsequent induction of adaptive immune responses. TLRs are involved in triggering intracellular signals, culminating in the activation of nuclear factor (NF)-κB, where it participates in enhancing expression of other immuneregulatory substances (Kawai and Akira 2006), whereas CAMs are known to mediate migration of cells to sites of inflammation.

Cytokines and cytokine receptors: Mediators regulating cancer cachexia are thought to be derived from immune or tumour cells, or the targeted tissues undergoing wasting (both adipose tissue and skeletal muscle). All cytokines play a role in the induction of immune and inflammatory responses. Consequences on cellular function are vast including, proliferation, mediating intracellular tissue cross talk, chemotaxis and killing. Genetic variants of genes encoding proinflammatory and antiinflammatory cytokines are presented in table 2 in electronic supplementary material.

Cytokines bind to their appropriate receptor to initiate a downstream cascade of intracellular signalling. Signalling leads to either potentiation of the signal and to production of similar or other cytokines and their receptors or to a suppression of the signal (Ihle *et al.* 1995). Since the last review, the gene encoding the IL-6R protein has been shown to play an important role in the mediation of IL-6 signalling, and a genetic variant in this gene has been shown to influence the C-reactive protein (CRP) level (Ridker *et al.* 2008). Genetic variants of genes encoding cytokine receptors and related binding proteins are presented in table 3 in electronic supplementary material.

Acute phase protein reactants: An organism responds to the presence of acute infection, tissue injury, trauma or surgery by mounting an acute phase response (APR); this is designed to help limit tissue injury by the increased synthesis of key defence / repair proteins by the liver. However, in certain circumstances, when dietary protein intake is limited and the APR is prolonged or severe, an APR can exacerbate muscle wasting by increasing the demands for certain amino acids to support increased hepatic export protein synthesis. They have also been shown to be predictors of adverse outcomes in cancer patients (Stephens *et al.* 2008). Some acute phase proteins also have roles in modulating immune response such as CRP. CRP genetic variants have been increasingly studied since the last review and some of these have been replicated

in other studies. These represent promising targets for future genetic studies on links with degrees of cachexia. Variants in genes coding for APPR are shown in table 4 in electronic supplementary material.

Central homeostasis

Energy production: The human body responds to stress with dramatic resilience and ultimately aims to maintain homeostasis. The mechanisms to respond to injury can ultimately prove detrimental to the host. For example, although the hepatic acute phase protein response is useful in acute injury (e.g. haemostasis and wound healing), if the response is prolonged and potentially futile (as in advanced cancer), then what results is an accelerated loss of skeletal muscle and excess morbidity and mortality. In certain forms of cancer, patients with cachexia have been observed to have much higher resting energy expenditure (REE) (Fredrix *et al*. 1991). Gene polymorphisms in the regulatory pathways controlling energy intake and expenditure are discussed here. The following section also explores genes involved in growth and development, and metabolic pathways common to both muscle and adipose tissues.

Electronic supplementary table 5 shows the genes involved in energy production, these consist mainly the uncoupling proteins. These are transporters present in the mitochondrial inner membrane that mediate a regulated discharge of the protein gradient that is generated by the respiratory chain. This serves to regulate functions such as thermogenesis, maintenance of the redox balance, or reduction in the production of reactive oxygen species (Ledesma *et al.* 2002). No new functional SNPs were found in this group during this review.

Insulin-like growth factors and related proteins: The IGF signalling pathway consists of two main ligands (IGF-1 and IGF-2), two cell surface receptors (IGFR1 and IGFR2), and six high affinity IGF-binding proteins (IGFBP1–6) (Jones and Clemmons 1995). The predominant regulator of skeletal muscle hypertrophy is through stimulation of the PI3K/AKT pathway by insulin or IGF-1 (Bodine *et al.* 2001a, b; Rommel *et al.* 2001; Glass 2010). Mice in which AKT is transgenically expressed and inducibly activated in skeletal muscle demonstrate dramatic hypertrophy upon the activation signal (Pallafacchina *et al.* 2002; Lai *et al.* 2004; Izumiya *et al.* 2008), helping to prove that AKT is the pathway that is sufficient to mediate hypertrophy downstream of IGF1 upregulation. Activation of AKT leads to an increase in the mTOR/p70S6K pathways and a rise in protein synthesis. As well as inducing protein synthesis, IGF1 can inhibit skeletal muscle atrophy. In the presence of upregulated IGF1 signalling, the atrophy genes *MuRF*-1 and *MAFbx*/*atrogin*-1 are actively inhibited (Bodine *et al.* 2001a, b). A number of new genetic variants for IGF-1 and IGF-1R have been discovered recently and are included (table 6 in electronic supplementary material).

Corticosteroid signalling proteins: Corticosteroids are essential steroid hormones that are secreted by the adrenal cortex and affect multiple organ systems. Corticosteroids are involved in a wide range of physiologic systems such as stress response, immune response and regulation of inflammation, carbohydrate metabolism and protein catabolism. A number of new genes have been included, the corticotropin-releasing hormone receptor 1 (CRHR1) binds to corticotropin-releasing hormone, a potent mediator of endocrine, autonomic, behavioural and immune responses to stress. A number of polymorphisms in this gene have been shown to effect circulating levels of CRP and ICAM-1 (Wilker *et al.* 2009). The glucokinase regulatory protein (GCKR) binds and moves glucokinase (GK); thereby, controlling both activity and intracellular location (Van Schaftingen 1994) of this key enzyme of glucose metabolism (Iynedjian 2009). Genetic variants of this protein have also been linked with alterations in circulating CRP levels (Ridker *et al.* 2008). The genetic variants of the components in the mechanism of corticosteroid signalling are examined in table 7 in electronic supplementary material.

Muscle

In healthy adults, skeletal muscle mass is maintained within relatively narrow limits, reflecting a dynamic balance between protein synthesis and degradation. A predominance of either will result in muscle hypertrophy or atrophy. Even small changes in protein synthesis or degradation will lead to large protein deficits over time due to the continuous process of protein turnover. In cancer cachexia, there is ongoing debate as to whether a reduction in protein synthesis, an increase in protein degradation or a combination of both is more relevant. Although there is depletion of both adipose tissue and lean body mass, it is skeletal muscle loss that has the greatest impact on patients' function and quality of life and is clearly associated with a poor outcome (Tan *et al.* 2009; Fearon *et al.* 2011; Miller *et al.* 2012). Highlighted here are genetic variations that affect the structure and function of muscle as well as those that regulate muscle synthesis and degradation.

Muscle structure and function: *IL-15* signals through IL-15 receptor alpha (IL-15RA) and is found in abundance in skeletal muscle. *IL-15* is shown to be anabolic, marked by an increase in myosin heavy chain accumulation (Quinn *et al.* 2002). ACTN3 (alpha-actinin 3) binds to actin at the Z-line within muscle fibres and acts to anchor actin filaments. Polymorphisms in ACTN3, IL15 and IL15RA are shown in table 8 in electronic supplementary material.

Recent studies have focussed on polymorphisms associated with alterations of fat free mass in the gene encoding the vitamin D receptor (VDR). Promising new variants encoding for this protein are also included in table 8 in electronic supplementary material. Inhibin β C (*INHBC*) is

a newly added gene, it is part of the transforming growth factor β pathway regulating myostatin (a negative regulator of muscle mass). Polymorphisms identified in this gene are shown in table 8 in electronic supplementary material. Another significant new addition to the genes implicated in cancer cachexia is thyrotropin-releasing hormone receptor (TRHR). Thyrotropin-releasing hormone is released by the anterior pituitary and acts on a number of tissues, including muscle to influence metabolic rates. Genetic variants are listed in table 8 in electronic supplementary material.

New variants in the *ACE* gene have also been included. Alterations in this gene have led to differences in oxygen carrying capacity of muscles as well as increased vasoconstriction in blood vessels within the muscle architecture (Costa *et al.* 2009). Acute and chronic exposure to angiotensin II in animal models are associated with weight loss and enhanced protein breakdown in skeletal muscle (Brink *et al.* 2001). Genetic variants are listed in table 8 in electronic supplementary material.

Muscle synthesis: The main signalling pathway for muscle synthesis is via the IGF-1/PI3K/AKT axis. Polymorphisms relating to these can be found in the insulin-like growth factors and related proteins section (table 6 in electronic supplementary material). mTOR, RUNX1, phosphoinositide 3-kinase (PIK3) and various isoforms have been implicated in hypertrophy signals of muscle mass (Bonetto *et al*. 2011) and have been added to the list of genes. However very few studies have looked into how variants effect structure and function of these gene products. A number of genes from expression arrays were also included as *cis* acting or *trans* acting polymorphisms on the genome may be affecting their expression. Genetic variants influencing function can be found in table 9 in electronic supplementary material.

Muscle proteolysis: In atrophying muscles, the ubiquitin ligases, MuRF1/atrogin-1 and MAFbx, are induced and this response is necessary for rapid atrophy. FOXO isoforms 1 and 3 are known to act on MuRF1 promoter to cause MuRF1 transcription and this leads to dramatic atrophy of myotubes and muscle fibres (Sandri *et al*. 2004; Bonetto *et al*. 2011). IKKα also influences the ubiquitin proteasome pathway (UPP) pathway and a number of functional polymorphisms are listed in table 10 in electronic supplementary material. Another gene encoding for tumour necrosis factor receptor (TNFR) associated factor 6 (TRAF6) is an important adaptor protein involved in receptor mediated activation of various signalling pathways in response to cytokines and bacterial products. TRAF6 also possesses E3 ubiquitin ligase activity causing lysine-63 linked polyubiquitination of target proteins (Paul and Kumar 2011).

A more recent factor, which has captured the attention of many investigators, is the TGF-β family member, myostatin. Genetic null animals for myostatin, demonstrate dramatic muscle hypertrophy (Mosher *et al.* 2007). Myostatin is synthesised and secreted mainly from skeletal muscle cells. Myostatin acts firstly by signalling through the activin type II receptor (ACTRIIB), which then recruits an Alk family kinase, resulting in the activation of a SMAD2 and SMAD3 transcription factor complex (Sartori *et al.* 2009; Trendelenburg *et al.* 2009).

STAT3 has recently been shown to influence muscle wasting by altering the profile of genes expressed and translated in muscle such that amino acids liberated by increased proteolysis in cachexia are synthesized into acute phase proteins and exported into the blood (Bonetto *et al.* 2011).

Peroxisome proliferator activated receptor gamma (PPARγ) and its response gene, Acyl CoA synthetase 5 (*ACSL5*), have an important role in fatty acid metabolism and may affect weight loss in response to caloric restriction. Genetic polymorphisms have demonstrated reduced ACSL5 mRNA in skeletal muscle biopsies (Adamo *et al.* 2007). Polymorphisms in all these genes are presented in table 10 in electronic supplementary material.

Adipose tissue

Incidence of obesity and diabetes continues to increase worldwide. The obese patient is generating a new challenge in medicine and treatment. Physiology of obese patients differs remarkably from a normal weighted individual. Recently, patients suffering from advanced cancer have been found to be overweight rather than underweight (Irigaray *et al.* 2007). This has been shown to confound conventional measurements for risk stratification such as body mass index (BMI). A recent study of pancreatic cancer patients has shown that severe muscle depletion when combined with obesity to be an independent adverse prognostic indicator in this patient group and should be considered as an alternative and more powerful means of risk stratification (Tan *et al.* 2009). It is however unclear how muscle depletion combined with overweight/obesity causes accelerated demise. The adipokines secreted by excess adipose tissue may act as systemic inflammatory mediators, inducing insulin resistance in skeletal muscle and leading to a further increase in muscle protein loss. Increased lipolysis appears to be a key factor underlying fat loss, though decreases in lipid deposition and adipocyte development may also contribute (Legaspi *et al.* 1987). The following section examines polymorphisms in genes regulating adipose tissue metabolism.

Adipogenesis: Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARα is known to participate in the regulation of key proteins involved in extracellular lipid metabolism, fatty acid oxidation and inflammation (Torra *et al.* 2001). PPARγ is an important regulator of fat cell function and involved in differentiation of new adipocytes and by inducing expression of genes promoting uptake of fatty acids,

triglyceride synthesis and insulin sensitivity (Lehrke and Lazar 2005). Lipin proteins (lipin-1, lipin-2 and lipin-3) also act as transcriptional coactivators that regulate expression of lipid metabolism genes (Reue 2009). Polymorphisms in these genes are shown in table 11 in electronic supplementary material.

Lipid turnover and transport: Lipid metabolism protein apolipoprotein C-III (apoC-III) inhibits triglyceride hydrolysis. Genetic variants have been shown to have lower fasting and postprandial serum triglycerides, higher levels of HDL-cholesterol and lower levels of LDL-cholesterol (Pollin *et al.* 2008). A genetic link between lipid metabolism and inflammation has been suggested by the association between variation in the *APOE* gene and plasma CRP. A variant in the *LRRFIP1* gene, which has been implicated in TNFa expression has been shown to be associated with adiposity and inflammation (Plourde *et al.* 2012).

A recent experiment in tumour bearing mice demonstrated during the early and intermediate phases of tumour growth and cachexia, food intake remained normal while plasma levels of proinflammatory cytokines and zinc-α2-glycoprotein rose. The investigators found that genetic ablation of adipose triglyceride lipase (ATGL) prevented an increase in lipolysis and the net mobilization of adipose tissue associated with tumour growth. Unexpectedly, they also observed that skeletal muscle mass was preserved and that activation of proteasomal degradation and apoptotic pathways in muscle was averted. Ablation of hormone sensitive lipase (HSL) had similar but weaker effects. Genetic variants in both these new genes were explored and are listed in table 12 in electronic supplementary material.

Zinc-α-2-glycoprotein (ZAG), otherwise known as LMF, is involved in the specific mobilization of adipose tissue, with increased oxidation of released fatty acids, possibly via induction of uncoupling protein (UCP) expression (Bing *et al.* 2002). ZAG isolated from the MAC16 murine tumour, or from the urine of patents with cancer cachexia, stimulates lipolysis directly through interaction with adenylate cyclase in a guanosine triphosphate (GTP)-dependent process (Bing *et al.* 2004; Bao *et al.* 2005). A polymorphism associated with change of function is shown in table 12 in electronic supplementary material.

Adipokines and adipokine receptors: Adipose tissue, similar to skeletal muscle is an active metabolic and endocrine organ. A number of inflammatory cytokines secreted by adipose tissue have been shown with varying effect to influence the development of diseases such as insulin resistance, diabetes and cancer cachexia by acting on muscle and fat metabolism (Fantuzzi and Faggioni 2000). These adipokines act locally in an autocrine/paracrine manner and/or as endocrine signals to regulate appetite, energy expenditure and a range of physiological processes including insulin sensitivity and inflammatory response which may play an important role in the pathogenesis of cancer cachexia (Kerem *et al.* 2008). Resistin is an adipokine which appears to have effects on substrate metabolism through impairment of insulin action and insulin independent pathways (McTernan *et al.* 2006). Polymorphisms within the *RETN* gene which codes for resistin that may influence the development of cachexia (table 13 in electronic supplementary material).

Adiponectin is secreted from adipose tissue and binds to a number of receptors including adiponectin receptors 1 and 2. Adiponectin is produced by the adipocyte and has been shown to decrease insulin resistance. Unlike other adipokines associated with chronic inflammation, adiponectin is inversely related to insulin resistance and BMI. It appears to have protective metabolic and antiinflammatory properties (Marcell *et al.* 2005).

The adipokine, leptin plays a key role in regulating energy intake and energy expenditure, including appetite and metabolism. Leptin acts through the leptin receptor. Polymorphisms in genes coding for adiponectin, leptin and their respective receptors are given in table 13 in electronic supplementary material.

Appetite

Muscle mass is clearly sensitive to food intake. The pathogenesis of cancer anorexia is multifactorial and reflects the complexity of the mechanisms controlling energy homeostasis under physiological conditions. The main molecular mechanisms regulating the cancer anorexia–cachexia syndrome include alterations in brain neurochemistry. In particular, the hypothalamic melanocortin system appears not to respond appropriately to peripheral inputs, and its activity is diverted largely towards the promotion of catabolic stimuli promoting metabolism of carbohydrates, lipids, and proteins in peripheral tissues leading to insulin resistance, increased lipolysis and accelerated muscle proteolysis (Tisdale 2002). Ghrelin is produced by the P/D1 cells of the stomach and acts as the natural counterpart to leptin. Ghrelin exerts its effects by promoting food intake (via the orexigenic neuropeptide Y(NPY) system) (Toshinai *et al.* 2003) and decreases sympathetic nerve activity (Matsumura *et al.* 2002). The melanocortin receptors, a family of G-protein coupled receptors, bind α-melanocyte stimulating hormone (α-MSH). Melanocortin 3 and 4 receptors have been found to be involved in feeding behaviour and regulation of metabolism (Fan *et al.* 1997).

NPY acts as a neurotransmitter in the brain and in the autonomic nervous system. An increase in NPY signalling leads to increased food intake (Hanson and Dallman 1995). Genetic variants have been added to this new gene and are listed in table 14 in electronic supplementary material.

Others

Metallothionein (MT) is a family of cysteine-rich, low molecular weight proteins. MTs have the capacity to bind both physiological (such as zinc, copper and selenium) and

xenobiotic (such as cadmium, mercury, silver and arsenic) heavy metals through the thiol group of its cysteine residues, which represents nearly 30% of its amino acidic residues. Zinc homeostasis is often disrupted in cancer cachexia. It has been proposed that the acute phase response can mediate zinc redistribution and accumulation in skeletal muscle tissue and contribute to the activity of the UPP that regulates protein catabolism (Siren and Siren 2010).

P2Y-receptors belong to the superfamily of G-protein coupled receptors and mediate the actions of extracellular nucleotides in cell to cell signalling. The P2Y11 receptor is highly expressed in immunocytes and may play a role in the differentiation of these cells (von Kugelgen 2006). Genetic variants encoding the proteins discussed above may play a role in the development of cachexia and are listed in table 15 in electronic supplementary material.

A number of new genes and SNPs are listed in table 15 in electronic supplementary material, these have evolved from a recent candidate gene approach study identifying SNPs in cancer cachexia. Target SNPs identified from the previous review which could not be genotyped in a cancer population due to experimental design were substituted for the nearest SNP with a linkage disequilibrium of >0.9. These SNPs have been added to the current review as they have been genotyped in patients with cancer cachexia.

Analysis of results

Clinical significance

Clinical significance is defined as any SNP affecting more than one of the recognized hallmarks of cancer cachexia. Inflammation has been shown to influence the severity of cancer cachexia and was therefore identified as a clinical feature. Changes in body composition of muscle and/or fat mass form the basis of cancer cachexia; therefore, SNPs that have the potential to alter an individual's body composition, whether to increase or decrease these components will play a significant clinical role in the development of cancer cachexia or not. Lastly, any SNPs influencing overall survival were included in a definition of clinical significance.

Out of 281 candidate polymorphisms that were identified and summarized here, the functional or clinical significance of 80 polymorphisms have been verified in more than one study. Of these 80 polymorphisms, 24 have been shown to have more than one effect on clinical features associated with cancer cachexia (i.e. inflammation, changes in lean and/or fat mass, and overall survival), these are termed promising SNPs. An indepth analysis of the 24 promising SNPs as biomarkers for susceptibility of cancer cachexia (table 2) is presented below.

(i, ii) The G allele of TLR-1 (−7202A/G) (rs5743551) is associated with elevated TLR1-mediated cytokine production (Wurfel *et al.* 2008; Pino-Yanes *et al.* 2010). TLR1 (−7202G) marks a coding SNP that causes higher TLR1-induced NF-kB activation and higher cell surface TLR1 expression (Wurfel *et al.* 2008). Toll-like receptor (TLR) pathways are critical components of the immune response to pathogens and disease (Trinchieri and Sher 2007). This particular polymorphism has been shown to lead to decreased survival in patients with sepsis (Wurfel *et al.* 2008) and NSCLC (Dai *et al.* 2012). In addition to this SNP in TLR-1, patients carrying the mutant allele T of TLR4 1196C/T (Thr399Ile, rs4986791) had lower TNF-α and sTNFR2 levels compared to patients carrying wild-type alleles (Jermendy *et al.* 2010). These patients carrying the mutant phenotype have also been shown to have increased total body fat, visceral fat, liver fat and decreased insulin sensitivity (Weyrich *et al.* 2010).

(iii) Steps in the inflammatory process include accumulation of lipids, recruitment of leucocytes and smooth muscle cells into vessel walls, and accumulation of extracellular matrix. Intercellular adhesion molecule-1 (ICAM-1) is integral in these cellular processes as interactions between ICAM-1 and activated receptors on the leucocytes result in firm adhesion and transmigration of leucocytes into the basement membrane of the vasculature. The T allele of rs5491 encodes a lysine to methionine substitution in exon 2 in the N-terminal domain of ICAM-1 and results in a protein that is unable to bind to fibrinogen and has a decreased affinity for T cells at lower ICAM-1 concentrations compared to wild-type ICAM-1 (Craig *et al.* 2000). This lead to increased circulating levels of sICAM-1 and an association with insulin resistance and the metabolic syndrome (Hsu *et al.* 2010).

(iv) The C allele of the A37674C *SELP* polymorphism (rs6136) is associated with decreased serum P selectin levels (Miller *et al*. 2004; Volcik *et al*. 2006). P selectin is required for efficient recruitment of neutrophils in acute inflammation and of macrophages in later stages of the inflammatory response and serum levels of P selectin have been found to be significant prognostic factors in survival in patients with gastric and colorectal malignancies (Alexiou *et al.* 2001, 2003). Patients with cancer who carry the C allele of the rs6136 polymorphism in *SELP* gene are at reduced risk of developing cachexia as defined by weight loss >10% (Tan *et al.* 2012).

(v–vii) TNF-α is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. The –308A allele (rs1800629) has been associated with an increased TNF-α production as well as a six-fold increase in transcription of the *TNF* gene (Wilson *et al.* 1997; Sallakci *et al.* 2005). Women carrying the A/A genotype have been linked to increased fat accumulation (Hoffstedt *et al.* 2000). The −863A allele (rs1800630) associated with decreased transcriptional activity and reduced serum TNF-α levels (Day *et al.* 1998; Skoog *et al.* 1999; Kaluza *et al.* 2000; Sharma *et al.* 2006).

Table 2. Polymorphisms replicated in more than one study and with at least two effects on clinical features associated with cancer cachexia ($n = 24$).

Gene	SNP	Previous S/R	New S/R	Functional Significance	Ancestal allele	SNP allele/s	MAF	SNP type	Systemic inflammation	BMI/ fat mass	Lean mass/ strength	Survival	Repeat studies
TLR-1	rs5743551		✓	The G allele of TLR-1 (-7202A/G) (rs5743551) is associated with elevated TLR1-mediated cytokine production (Wurfel *et al.* 2008) Alleles 7202G and 248Ser, and the 248Ser-602Ile haplotype were associated with circulatory dysfunction among severe septic patients ($0.001 \leq P \leq 0.022$), and with reduced IL-10 ($0.012 \leq P \leq 0.047$) and elevated CRP ($0.011 \leq P \leq 0.036$) serum levels during the first week of sepsis development (Pino-Yanes *et al.* 2010).	C	T	T = 47%	nearGene-5	↑Pino-Yanes *et al.* (2010)			↓Dai *et al.* (2012)	✓Pino-Yanes *et al.* (2010), Wurfel *et al.* (2008), Dai *et al.* (2012)
TLR-4	rs4986791		✓	Serum levels of TNF-alpha and its soluble receptors are elevated and associated with increasing BMI values in obese children. Serum cytokine levels, as modifying factors of insulin, resistance may be affected by TLR4 polymorphisms in obese children (Jermendy *et al.* 2010).	C	T	T = 3%	Missense Thr–Ile	↑Dhiman *et al.* (2008)	↑Weyrich *et al.* (2010)			✓van Rijn *et al.* (2008)
ICAM-1	rs5491		✓	ICAM1 DNA segment variants were associated with sICAM-1 protein level including the novel finding that levels differ by the functional variant rs5491 (Bielinski *et al.* 2011)	A	T	T = 7%	Missense Lys–Met	↑	↑Hsu *et al.* (2010)			✓Hsu *et al.* (2010)
SELP	rs6136	✓		Decreased serum P-selectin levels (Miller *et al.* 2004; Volcik *et al.* 2006) P-selectin genotype is associated with the development of cancer cachexia (Tan *et al.* 2012).	T	G	G = 4%	715 Missense Thr–Pro	↓Miller *et al.* (2004), Volcik *et al.* (2006)			↑	✓Tan *et al.* (2012), Miller *et al.* (2004), Volcik *et al.* (2006)
TNF-α	rs1800629	✓		Increased TNF-α production (Sallakci *et al.* 2005) Six-fold increase in transcription of TNF-α (Wilson *et al.* 1997)	G	A	A = 10%	nearGene-5	↑	↑			✓ Wilson *et al.* (1997), Sallakci *et al.* (2005)
	rs361525		✓	Decreased transcriptional activity (Kaluza *et al.* 2000) Decreased PMBC production of TNF-α after stimulation with T-cell mitogens (Kaluza *et al.* 2000) Decreased insulin resistance (Day *et al.* 1998)	G	A	A = 5%	nearGene-5	↓	↓			✓

Table 2 (*contd*)

Gene	SNP	Previous S/R	New S/R	Functional Significance	Ancestal allele	SNP allele/s	MAF	SNP type	Systemic inflammation	BMI/ fat mass	Lean mass/ strength	Survival	Repeat studies
	rs1800630	✓		Reduced total serum IgE levels (Sharma *et al.* 2006) Reduced serum TNF-α levels (Sharma *et al.* 2006) 31% decrease in transcription of *TNF*-α (Skoog *et al.* 1999)	C	A	A = 15%	nearGene-5	↓	↓			✓
LTA	rs909253	✓		Increased serum TNF-α levels (Stuber *et al.* 1996; McArthur *et al.* 2002)	A	G	G = 40%	Intron	↑			↓	✓
*IL-1*β	rs1143627	✓		Increased expression of *IL-1*β gene with T allele (Lind *et al.* 2007) Increased IL-1β production from whole blood eukocytes after stimulation with LPS with -31T/-l511C/-1470G haplotype (Wen *et al.* 2006) Increased transcriptional activity with −31T/-511C/-1470G haplotype (Wen *et al.* 2006)	G	A	G = 48%	nearGene-5	↑			↑	✓
	rs16944	✓		Increased IL-1β production from whole blood leukocytes after stimulation with LPS with -31T/-511C/-1470G haplotype (Wen *et al.* 2006) Increased transcriptional activity with -31T/-511C/-1470G haplotype (Wen *et al.* 2006) No significant increase in IL-1β production in response to LPS in patients homozygous for T allele Awomoyi *et al.* (2005)	A	G	A = 48%	nearGene-5	↓			↓	✓
	rs1143634	✓		T/T genotype associated with lower plasma levels of IL1-RA (Tolusso *et al.* 2006) Increased human amniochorion IL-1β production after stimulation with LPS (Hernandez-Guerrero *et al.* 2003)	G	A	A = 15%	Synonymous Phe - Phe	↑	↓		↓	✓
IL-6	rs1800795	✓		Lower levels of IL-6 in plasma in healthy subjects (Fishman *et al.* 1998) Higher fasting plasma insulin levels with *G* allele (Yang *et al.* 2005) Lower circulating adiponectin levels with *G* allele (Yang *et al.* 2005)	G	C	C = 19%	nearGene-5	↓	↑	↓		✓
IL-18	rs549908		✓	Increased IL-18 production from LPS and A23187 + PMA stimulated monocytes in 105AA and -137GG (Arimitsu *et al.* 2006) Haplotype of common alleles (GTATA) associated with significantly lower IL-18 (Thompson *et al.* 2007)	T	G	G = 23%	Synonymous Ser - Ser	↑	↓			✓ Arimitsu *et al.* (2006), Thompson *et al.* (2007)

Table 2 (*contd*)

Gene	SNP	Previous S/R	New S/R	Functional Significance	Ancestal allele	SNP allele/s	MAF	SNP type	Systemic inflammation	BMI/ fat mass	Lean mass/ strength	Survival	Repeat studies
IGF-1	rs7136446		✓	Genotype CC of rs7136446 associated with higher body fat and increased maximal force production (Huuskonen *et al.* 2011) Significantly associated with elevated levels of IGF-I (Verheus *et al.* 2008)	T	C	C = 29%	Intron	↑	↑	↑		✓ Verheus *et al.* (2008), Huuskonen *et al.* (2011),
NR3C1	rs6195		✓	Reduced first phase glucose stimulated insulin secretion and disposition index in women, but not in men (van Raalte *et al.* 2012). Associated with enhanced glucocorticoid sensitivity (Russcher *et al.* 2005).	A	G	G = 5%	Missense Asn–Ser	↓	↓			✓ van Raalte *et al.* (2012), Jewell and Cidlowski 2007
GCKR	rs780094		✓	Highly associated with serum CRP levels (Ridker *et al.* 2008)	C	T	T = 39%	Intron	↑	↑			✓ Ridker *et al.* (2008), Stancakova *et al.* (2012)
CNTF	rs1800169		✓	G/A genotype possesses significantly greater muscular strength and muscle quality at relatively fast contraction speeds than do G/G individuals (Roth *et al.* 2001)	G	A	A = 12%	Intron		↑	↑		✓ Roth *et al.* (2001), Heidema *et al.* (2010)
ACSL5	rs2419621		✓	Associated with marked weight loss in dieting and increased levels of ACSL mRNA in skeletal muscle biopsies (Adamo *et al.* 2007; Teng *et al.* 2009)	C	T	T = 26%	nearGene-5		↓	↓		✓ Adamo *et al.* (2007, Teng *et al.* (2009)
LPL	rs328	✓		Significantly lower IL-8 levels (Ak *et al.* 2007) Increased LPL activity Kozaki *et al.* (1993; Groenemeijer *et al.* 1997)	C	G	G = 10%	Stop-Gain 447 Ser - XXX	↓	↓			✓
ZAG	rs4215		✓	GG genotype subjects in rs4215 site have an increased susceptibility to obesity when compared with the AA + AG genotype subjects (Zhu *et al.* 2012) rs4215 was associated with total cholesterol and LDL-C but not with HDL-C or TG (Olofsson *et al.* 2010)	G	A	A = 42%		↑	↑			✓ Zhu *et al.* (2012), Olofsson *et al.* (2010)
RETN	rs1862513	✓		Increased plasma resistin (Cho *et al.* (2004; Osawa *et al.* 2007)	G	C	G = 31%	U/K	↑	↑			✓
ADIPOQ	rs2241766	✓		Increased plasma adiponectin (Berthier *et al.* 2005; Mackevics 2006)	T	G	G = 14%	Cds – synon Gly–Gly		↓	↑		✓
ADIPOR2	rs767870		✓	Increased ADIPOR2 protein in monocytes (Halvatsiotis *et al.* 2010)	A	G	G = 25%	Intron	↑	↑			✓ Vaxillaire *et al.* (2006), Kotronen *et al.* (2009)

Table 2 (*contd*)

Gene	SNP	Previous S/R	New S/R	Functional Significance	Ancestal allele	SNP allele/s	MAF	SNP type	Systemic inflammation	BMI/ fat mass	Lean mass/ strength	Survival	Repeat studies
NPY	rs16139		✓	leucine7 to proline7 in the signal peptide of preproNPY has been associated with increased risk factor for many cardiovascular diseases such as accelerated atherosclerosis (Niskanen *et al.* 2000). Furthermore, elevated serum lipid levels have been associated with the *P7* allele in certain populations (Karvonen *et al.* 2001). Additionally, the L7P polymorphism has been shown to increase the risk of type II diabetes (T2D) as well as earlier onset of the disease and vascular complications (Jaakkola *et al.* 2006).	T	C	C = 6%	Missense Leu–Pro	↑	↑			✓ Karvonen *et al.* (2001), Jaakkola *et al.* (2006).

Obese people express 2.5-fold more TNF mRNA in fat tissue (Hotamisligil 1999). The −238A allele of the SNP rs361525 has shown decreased transcriptional activity of TNFα (Kaluza *et al.* 2000), as well as decreased peripheral mononuclear blood cells (PMBC) production of TNF-α after stimulation with T-cell mitogens (Kaluza *et al.* 2000). It has also been shown to decrease insulin resistance (Day *et al.* 1998).

(viii) LTA is part of the tumour necrosis factor family, it is produced by lymphocytes, and mediates inflammatory responses, stimulation of immune system, and apoptosis (Aggarwal *et al.* 1985). The G allele of the 252 A>G polymorphism (rs909253) has been associated with increased serum TNF-α levels (Stuber *et al.* 1996; McArthur *et al.* 2002), and patients who are A/A homozygotes have been linked with better prognosis in lung cancer and gastric cancer (Shimura *et al.* 1994, 1995).

(ix–xi) IL-1β is a product of the *IL1B* gene and plays a role in potentiating an inflammatory response. The −31 C>T (rs1143627) and −511 C>T (rs16944) polymorphisms in the promoter region of the *IL1B* gene have been linked with increased transcriptional activity of the *IL1B* gene and subsequently increased IL-1β production (Wen *et al.* 2006). These two alleles are also linked with poorer progression free survival and overall survival in advanced gastric cancer (Graziano *et al.* 2005). A synonymous C to T polymorphism at nucleotide position 3953 (rs1143634) has resulted in Increased IL-1β levels (Hernandez-Guerrero *et al.* 2003). The T/T genotype has also been associated with lower plasma levels of IL-1 receptor antagonist (IL-1RA) (Tolusso *et al.* 2006). In addition, the T allele has found to be a major risk factor for cachexia in gastric cancer (Zhang *et al.* 2007), as well as being linked to lower total fat mass (Strandberg *et al.* 2006). The T/T genotype was found to be associated with shorter survival in pancreatic cancer (Barber *et al.* 2000). All of which lead these three polymorphisms to be of particular interest in potential candidates for cancer cachexia.

(xii) IL-6 is a well characterized cytokine involved in a number of cellular functions. IL-6 mediates B cell differentiation and maturation, immunoglobulin secretion, cytotoxic T cell differentiation and acute-phase protein production (Kishimoto 2005). The −174 G>C promoter polymorphism (rs1800795) in the *IL6* gene has been associated with lower serum levels of IL-6 (Fishman *et al.* 1998). The G allele has been linked to higher fasting insulin and lower adiponectin levels which may have a role in the regulation of adiposity (Yang *et al.* 2005). In addition, the C/C genotype has been associated with lower fat free mass and increased waist circumference (Roth *et al.* 2003; Berthier *et al.* 2005).

(xiii) IL-18 is produced by macrophages and functions by binding to the interleukin 18 receptor inducing cell mediated immunity following infection. After stimulation with

IL-18, natural killer (NK) cells and certain T cells release interferon-γ (IFN-γ) that plays an important role in activating the macrophages. Apart from its physiological role, IL-18 is also able to induce severe inflammatory reactions. Individuals with the allele 105AA (rs549908) demonstrated increased IL-18 production from LPS and A23187 + PMA stimulated monocytes (Arimitsu *et al.* 2006). Haplotype of common alleles have been shown to be associated with significantly lower IL-18 (Thompson *et al.* 2007).

(xiv) IGF-1 is one of the most potent natural activators of the AKT signalling pathway which is the main stimulator of cell growth and multiplication. IGF-1 also mediates many of the growth-promoting effects of growth hormone (GH) (Jones and Clemmons 1995). The genotype CC of rs7136446 associated with higher body fat and increased maximal force production (Huuskonen *et al.* 2011), it has also been shown to be significantly associated with elevated levels of circulating IGF-I (Verheus *et al.* 2008).

(xv) The glucocorticoid receptor (GR, or GCR) also known as NR3C1 (nuclear receptor subfamily 3, group C, member 1) is the receptor to which cortisol and other glucocorticoids bind. In the absence of glucocorticoids, GR resides in the cytosol complexed with a variety of proteins including heat shock protein 90 (hsp90), heat shock protein 70 (hsp70) and the protein FKBP52 (FK506-binding protein 52) (Pratt *et al.* 2006). The endogenous glucocorticoid hormone cortisol diffuses through the cell membrane into the cytoplasm and binds to the GR resulting in release of heat shock proteins. Activated GR can bind to the transcription factor NF-κB and prevent it from upregulating target genes (Ray and Prefontaine 1994). The mutant allele of the N363S (rs6195) SNP enhances glucocorticoid sensitivity by increasing gene transcription (Russcher *et al.* 2005). Indeed, in various studies, a link was established between the N363S SNP and characteristics of a cushingoid phenotype, including increased BMI and waist circumference, dyslipidaemia and augmented fasting insulin levels, indicating reduced insulin sensitivity (Roussel *et al.* 2003; Manenschijn *et al.* 2009).

(xvi) The glucokinase regulatory protein (GKRP) also known as glucokinase (hexokinase 4) regulator (GCKR) is a protein produced in hepatocytes. GKRP binds glucokinase (GK), thereby controlling both activity and intracellular location of this key enzyme of glucose metabolism (Van Schaftingen 1994; de la Iglesia *et al.* 1999). The glucose increasing major C allele of rs780094 of GCKR has been shown to be significantly associated with increased insulin resistance leading to development of T2DM and altered lipid metabolism (Stancakova *et al.* 2012; Li *et al.* 2013).

(xvii) CNTF is involved in the neuroendocrine signalling of appetite. It leads to marked weight loss through suppressed food intake without causing hunger or stress (Lambert *et al.* 2001). CNTF receptor-α is abundantly expressed in skeletal muscle (Ip *et al.* 1993; Fraysse *et al.* 2000). As such, recent studies have examined the roles of CNTF and CNTF genotype on neuromuscular disease and muscle function. CNTF administration has been shown to prevent losses of soleus muscle mass and function after hindlimb suspension in rats (Fraysse *et al.* 2000). In humans, the A allele mutation of rs1800169 possesses significantly greater muscular strength and muscle quality at relatively fast contraction speed than the ancestral G allele individual (Roth *et al.* 2001). This polymorphism has also been associated with a global weight gain in healthy humans (Heidema *et al.* 2010).

(xviii) Uptake of FFA by skeletal muscle for metabolism is initiated by transmembrane acyl-CoA synthetase long-chain (ACSL) proteins that esterify FFAs to acyl-coenzyme A (acyl-CoA) molecules. Acyl-CoA species are used mainly in both the synthesis of cellular lipids and the degradation of fatty acids via β-oxidation. Small increases in the expression of ACSL5 in skeletal muscle could have profound effects on FFA utilization (Teng *et al.* 2009). A strong association between the common SNP rs2419621 and rapid weight loss in obese Caucasian females in response to restricted diet has been demonstrated (Adamo *et al.* 2007). The SNP located 12 nucleotides upstream of the second transcription start site of the *ACSL5* gene is characterized by a cytosine (rs2419621) to thymine (rs2419621) transition. This study also demonstrated that the T allele is associated with a 2.2-fold increase of ACSL5 transcript level in skeletal muscle biopsies when compared to noncarriers (Adamo *et al*. 2007). In a further study T allele variants were shown to create a functional *cis*-regulatory E-box element (CANNTG) that is recognized by the myogenic regulatory factor MyoD. The T allele promoted MyoD-dependent activation of a 1089 base pair *ACSL5* promoter fragment in nonmuscle CV1 cells. Differentiation of skeletal myoblasts significantly elevated expression of the *ACSL5* promoter. The T allele variants sustained promoter activity 48 h after differentiation, whereas the C allele variants showed a significant decline. These results revealed a mechanism for elevated transcription of *ACSL5* in skeletal muscle of carriers of the rs2419621 (T) allele, associated with more rapid diet-induced weight loss. This is the first example of a MyoD-binding polymorphism conferring differential promoter activity of a metabolic gene (Teng *et al.* 2009).

(xix) Lipoprotein lipase (LPL) plays a central role in the overall lipid metabolism and transport (Mead *et al.* 2002). The rs328 polymorphism in the *LPL* gene leads to a premature stop codon at amino acid 447. The stop codon results in lower LPL activity (Kozaki *et al.* 1993; Groenemeijer *et al.* 1997), and is associated with lower levels of IL-8 (Ak *et al.* 2007). Individuals not in possession of the stop codon are associated with central obesity (Huang *et al.* 2006).

(xx) Zinc-α-2-glycoprotein (ZAG), otherwise known as LMF, is involved in the specific mobilization of adipose tissue, with increased oxidation of released fatty acids, possibly via induction of uncoupling protein (UCP) expression (Bing *et al.* 2002). LMF isolated from the MAC16 murine tumour, or from the urine of patents with cancer cachexia, stimulated lipolysis directly through interaction with adenylate cyclase in a guanosine triphosphate (GTP) dependent process (Bing *et al.* 2004; Bao *et al.* 2005). This effect was also produced by the interaction of LMF with the β_3-adrenoceptor (Russell *et al.* 2002). Genotypes of rs4215 in *ZAG* gene have been suggested to be significantly associated with obesity. The GG genotype subjects in rs4215 site have an increased susceptibility to obesity when compared with the AA+AG genotype subjects (Zhu *et al.* 2012). In a separate study variations in the rs4215 genotype have been linked with changes in circulating levels of total cholesterol and LDL-C.

(xxi) The adipokine resistin potentiates a proinflammatory state, resistin also appears to have effects on substrate metabolism through impairment of insulin action and insulin independent pathways (McTernan *et al.* 2006). The –420 C>G polymorphism (rs1862513) is shown to be linked to increased plasma resistin (Cho *et al.* 2004; Osawa *et al.* 2007), and individuals with the G/G genotype are associated with an increased prevalence of obesity (Norata *et al*. 2007). Overall, increased plasma resistin has shown to correlate with increased CRP and insulin resistance (Degawa-Yamauchi *et al.* 2003; Silswal *et al.* 2005; Nagaev *et al.* 2006; Kusminski *et al.* 2007; Osawa *et al.* 2007).

(xxii, xxiii) Adiponectin is a protein hormone that is exclusively secreted from adipose tissue and modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism (Diez and Iglesias 2003). The *ADIPOQ* gene, which codes adiponectin, has a 45 T>G polymorphism (rs2241766) that is associated with increased plasma adiponectin (Berthier *et al.* 2005; Mackevics *et al.* 2006). Individuals with G/G genotype have been observed to be leaner with less abdominal fat (Loos *et al.* 2007). The adiponectin receptors ADIPOR2, serves as a receptor for adiponectin and mediates increased AMPK and PPAR-α ligand activities, as well as fatty acid oxidation and glucose uptake by adiponectin (Yamauchi *et al.* 2003). In peripheral monocytes, carriers of the major A allele (homozygotes and heterozygotes) of rs767870 polymorphism had higher levels of ADIPOR2 protein expression compared to homozygotes of the minor G allele (Halvatsiotis *et al.* 2010). This same SNP has also been associated with liver fat content and the incidence of type II DM (Vaxillaire *et al.* 2006; Kotronen *et al.* 2009).

(xxiv) Proinflammatory cytokines (TNFα and IL-1β) and hypothalamic serotonergic neurons have been implicated in the dysfunction of the hypothalamic melanocortin system (Inui 1999). The orexigenic neuropeptide Y (NPY) peptide system appears to be strongly influential in the control of feeding (Plata-Salaman 2000). The pathway originates in the hypothalamic arcuate nucleus (ARC) and extends projections widely over the brain (Plata-Salaman 2000). The role of cytokines in cancer anorexia may be affected through influence on the NPY system. The genetic variant rs16139 causing, leucine7 to proline7 in the signal peptide of pre-proNPY has been associated with increased risk factor for many cardiovascular diseases such as accelerated atherosclerosis (Niskanen *et al.* 2000). Further, elevated serum lipid levels have been associated with the P7 allele in certain populations (Karvonen *et al.* 2001). Additionally, the L7P polymorphism has been shown to increase the risk of type II diabetes as well as earlier onset of the disease and vascular complications (Jaakkola *et al.* 2006).

Pathway analysis

The 80 polymorphisms which have been verified in more than one study were found across 51 genes. These genes were entered into the IPA algorithm as focus genes and were found to be significantly interconnected in two major networks (table 3). The two networks are presented in figures 1 and 2.

Putative functions

Since gene association studies often identify surrogates for putative causal SNPs, it is imperative that data from selected SNPs be subjected to further analysis using prediction tools to shortlist candidates for finer analysis before causality could be established. Unless causative SNPs/genes are identified, development of targeted therapeutics are difficult to achieve. Of the 42 nonsynonymous polymorphisms entered into the SIFT algorithm (table 16 in electronic supplementary material) seven SNPs had a significant score ($P < 0.05$) causing an intolerant change of amino acid. Since SIFT programme only evaluates nonsynonymous SNPs, and the majority of polymorphisms lie within the regulatory regions at 3′, 5′ ends and in introns, we interrogated the SNPs selected using the SCAN database for insights into the potential contribution of selected SNPs on gene regulatory functions (*cis* effects or *trans* effects, eQTLs) (Hunter and Crawford 2008; Schadt *et al.* 2008; Fehrmann *et al.* 2011; Hao *et al.* 2012; He *et al.* 2013). We identified 132 SNPs as potential eQTLs in HapMap study populations (Caucasian, Yoruban, Han Chinese/Japanese) (table 17 in electronic supplementary material). Analysis of selected candidate SNPs for potential eQTLs revealed several target genes that are regulated both in *cis* and *trans*, as expected. Fine regulation in complex biological networks by a SNP may be direct or indirect, and likely influence gene expression through short or long range interactions. Prediction for regulation of expression was limited to those showing high statistical significance (P value$< 10^{-4}$) and to any one of

Table 3. Ingenuity pathway analysis of genes that were replicated in more than one study ($n = 51$).

Network	Molecules	Calculated score	Focus genes	Top functions of network
1	ADCY, **ADRB1, ADRB2, ADRB3**, beta arrestin, **CCL2**, **CRHR1**, cytochrome c, endothelin, estrogen receptor, G protein, G protein alphai, G protein β, GNRH, Gpcr, Gs-coupled receptor, **IGF1R**, IL-17f dimer, **IL-6R**, Jnk, **LEP, MC3R, MC4R,** Mmp, NADPH oxidase, **NPY,** P110, p85 (pik3r), Pdgfr, PLC, Shc, **TRHR,** trypsin, TSH, Voltage gated calcium channel	22	12	Cell signalling, neurological disease, nutritional disease
2	Three BETA HSD, **ADIPOQ, ADIPOR2,** Akt, AMPK, **AZGP1,** FKHR, **FOXO1, GHRL,** Gm-csf, HLA-DQ, Igf, **IGFBP3,** IL-8r, jINK1/2, MTORC1, Na+, K+ - ATPase, Nr1h, Pde4, PEPCK, **PIK3CB,** Ppp2c, PRKAA, Proinsulin, Ptk, Rab5, Rxr, Scavenger receptor class A, sPla2, T3-TR-RXR, thymidine kinase, **UCP2, UCP3, VDR,** vitaminD3-VDR-RXR	20	10	Endocrine system, development and function, carbohydrate metabolism, molecular transport

Focus genes are in bold. The software illustrates networks graphically and calculates a score for each network, which represents the approximate 'fit' between the eligible focus molecules and each network. The network score is based on the hypergeometric distribution and is reported as the –log (Fisher's exact test result).

the HapMap populations. Promising candidates from SCAN database should be further validated by independent methods (RT-PCR) to confirm for direction and magnitude of expression changes in a tissue-specific manner. In addition to pathway-based-candidate SNP approaches for cachexia, genomewide association studies (GWAS) have the potential to identify promising variants for further interrogation of the genome for genetic predisposition. International efforts are underway from our group to conduct large scale association studies using well defined phenotypes of cachexia and higher sample size to achieve the needed statistical power.

Discussion

Even with the same tumour type and burden, one individual may become cachectic, whereas another will not, such variation may relate to host genotype. Genetic variation in immunity and associated signalling pathways is known to relate to outcomes in major sepsis (Thair *et al.* 2011), and recent findings suggest a similar pattern in cancer cachexia (Tan *et al.* 2012). SNP in the IL-1, IL-6, and IL-10 genes that are linked to production rates of these cytokines have been associated with the prevalence of cachexia in gastric or pancreatic cancer (Tan and Fearon 2010). For example, the 1082G allele in the IL-10 promoter has been validated as a procachectic genotype in an independent cohort (Deans *et al.* 2009; Sun *et al.* 2010a, b). IL-10 has been shown to be elevated in a Myc/mTOR driven murine model of cancer cachexia (Robert *et al.* 2012), as well as in cachectic patients with colorectal cancer (Shibata *et al.* 1996). Others have identified associations with cachexia defined as >10% weight loss and polymorphisms in cytokine genes such as the IL1-B 3954C/T polymorphism (rs1143634) in patients with gastric cancer (Zhang *et al.* 2007). Cancer-related anorexia has been associated with the TNF-308G/A polymorphism (rs1800629) in patients with non-small cell lung cancer (Jatoi *et al.* 2010). Most recently, the C allele of the rs6136 polymorphism in the p-selectin gene has recently been associated with weight loss in a large heterogenous group of cancer patients and validated in an independent cohort (Tan *et al.* 2012). Taken together, these findings are consistent with a key role for the immune system in the variable presentation of cachexia. However, cancer cachexia has been defined in a number of ways (Bozzetti and Mariani 2009; Argiles *et al.* 2010; Muscaritoli *et al.* 2010; Argiles *et al.* 2011; Baracos 2011), mainly focussed on weight loss and the presence of systemic inflammation. This has led to phenotyping in many of the existing studies on genetic association relying wholly on a degree of weight loss and inflammation. However, one recent definition described cancer cachexia with a more musculocentric view (Fearon *et al.* 2011). This definition highlights the importance of skeletal muscle loss as one of the most significant events in cachexia and is associated with a poor

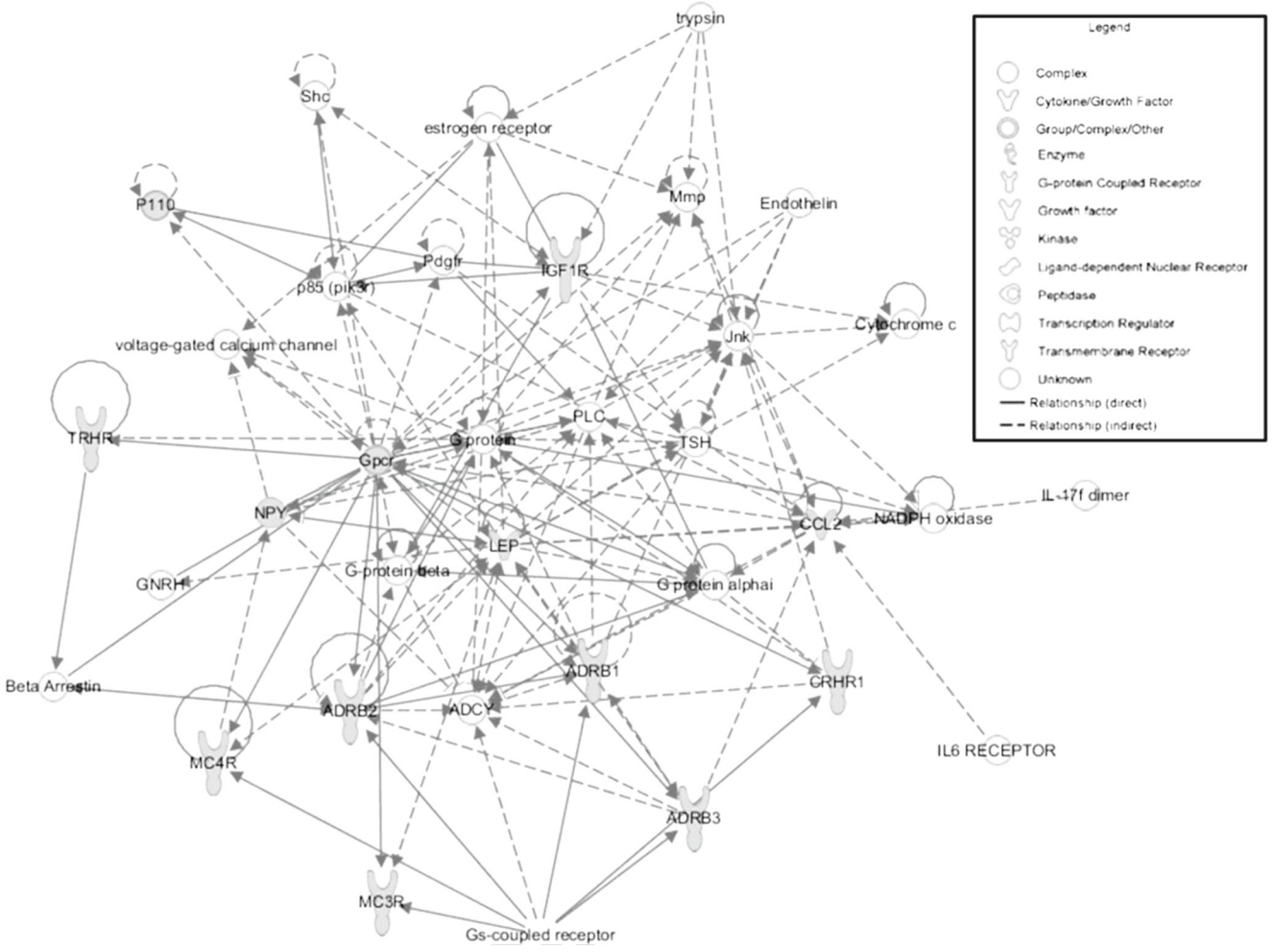

Figure 1. Connection map for first ranked network. Genes with variants that had functional or clinical associations replicated in at least one study were entered into the ingenuity pathway analysis software for an unsupervized functional analysis to discern regulatory networks that involved these molecules. Focus genes are shaded in grey. Solid lines show direct interaction (binding/physical contact); dashed lines show indirect interaction that is supported by the literature but possibly involving ≥1 intermediate molecules that have not been investigated definitively. Molecular interactions that involved only binding are connected with a line without an arrowhead because directionality cannot be inferred.

outcome (Tan *et al.* 2009; Fearon *et al.* 2011). In genetic studies of common diseases, the capacity to define genotypes is often far better than the capacity to define phenotypes; therefore, more robust classifications of phenotypes need to be sought. With the existing studies focussing mainly on weight loss and CRP to define cancer cachexia phenotypes this may have led to the discovery of associated SNPs exclusively involved in mediating the immune response. Recent advances in the use of routine CT scans in a cancer patient's care have led to unmasking of detailed body composition analysis for accurate measurements of skeletal muscle and adipose tissue mass (Prado *et al.* 2009). Taking into account, a level of skeletal muscle mass alongside degrees of weight loss and inflammation will improve accuracy of phenotyping and may open up the analysis to include genetic variants in muscle specific genes.

While some genetic variants can predisopose individuals to develop cancer cachexia, also there will be variants which may protect against the devlopment of the condition. In multigene studies, judicious selection of candidate genes and polymorphisms within them is a key element of study design. It is always important to choose genes products which interact within regulatory or metabolic pathways. In most cases, it is not realistic to analyse all possible gene variants and combinations; hence, existing polymorphisms should be initially prioritized on the basis of their likelihood to affect function of the encoded product (Tabor *et al.* 2002). Published studies linking genetic variation to cancer cachexia are listed in table 1, the definitions of the cancer cachexia phenotype vary greatly from weight loss cut-offs and inclusion of systemic inflammation, to performance status assessment and quality of life scores. The only study to directly state and include an independent validation cohort to confirm the presence of a significant SNP is the detection of the p-selectin genotype from our group (Tan *et al.* 2012). In the discovery cohort, a further 20 significant SNPs were

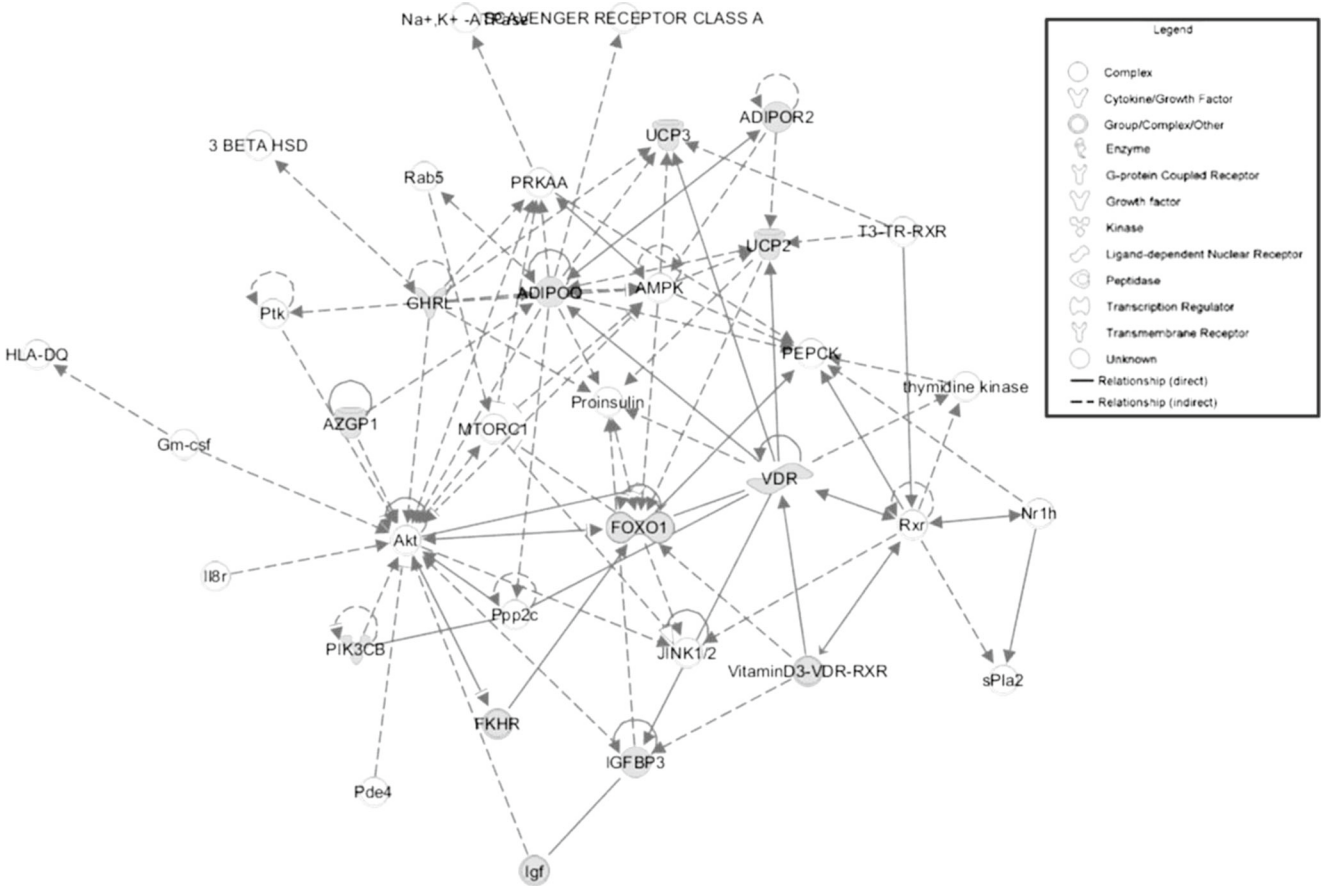

Figure 2. Connection map for second ranked network. Genes with variants that had functional or clinical associations replicated in at least one study were entered into the ingenuity pathway analysis software for an unsupervized functional analysis to discern regulatory networks that involved these molecules. Focus genes are shaded in grey. Solid lines show direct interaction (binding/physical contact); dashed lines show indirect interaction that is supported by the literature but possibly involving ≥1 intermediate molecules that have not been investigated definitively. Molecular interactions that involved only binding are connected with a line without an arrowhead because directionality cannot be inferred.

found; however, these did not reach significance in the validation cohort. These SNPs may be significant, but due to the size of the independent validation cohort, they failed to reach significance.

In the current review, functional polymorphisms in genes with a possible role in cachexia have been recorded as well as polymorphisms with clinical consequences related to cachexia such as inflammation, weight/body composition changes and cancer survival. Since cancer cachexia is a multifactorial disease involving a variety of biological pathways, it can be assumed that analysis of combinations of gene variants encoding interacting factors within a biological chain or cascade, rather than isolated investigation of its single components, may have more chances to reveal real causative connections between gene polymorphisms and phenotypes. Of the 80 polymorphisms with a potential role in the development of cachexia that have been independently verified in at least one repeat study, 24 polymorphisms have been shown to have more than one effect on clinical features associated with cancer cachexia. These 24 polymorphisms are likely to be the most promising candidates in terms of susceptibility biomarkers of cancer cachexia and should be further investigated.

Eighty-eight newly identified genes with a role in cancer cachexia were included since the last review. However, the main limitation to identifying new SNPs in these genes was the lack of studies to date into functional polymorphisms within them. Undertaking a GWAS would be one way to overcome this potential limitation and in the future would be preferable to repeating candidate gene selection studies, however without an adequate sample size and highly accurate phenotyping, coupled with lack of government funding to complete the project, candidate gene association studies will continue to provide novel insights into the genetics of cancer cachexia.

In conclusion, the current review has expanded on an initial framework to further enhance the possibility of identifying functional polymorphisms involved in cancer cachexia. Based on the expansion of the definition of cancer cachexia along with an inclusion of skeletal muscle mass and not just

weight loss and systemic inflammation, new muscle specific SNPs may provide novel biomarkers in the early detection of individuals at risk of developing cancer cachexia.

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