REVIEW ARTICLE

Identification of possible genetic polymorphisms involved in cancer cachexia: a systematic review

BENJAMIN H. L. TAN[1]*, JAMES A. ROSS[1], STEIN KAASA[2], FRANK SKORPEN[3], KENNETH C. H. FEARON[1] and EUROPEAN PALLIATIVE CARE RESEARCH COLLABORATIVE

[1]*Clinical and Surgical Sciences (Surgery), Royal Infirmary, University of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, UK*

[2]*Clinical Department of Cancer Research and Molecular Medicine, and* [3]*Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim N-7006, Norway*

Abstract

Cancer cachexia is a polygenic and complex syndrome. Genetic variations in regulation of the inflammatory response, muscle and fat metabolic pathways, and pathways in appetite regulation are likely to contribute to the susceptibility or resistance to developing cancer cachexia. A systematic search of Medline and EmBase databases, covering 1986–2008 was performed for potential candidate genes/genetic polymorphisms relating to cancer cachexia. Related genes were then identified using pathway functional analysis software. All candidate genes were reviewed for functional polymorphisms or clinically significant polymorphisms associated with cachexia using the OMIM and GeneRIF databases. Genes with variants which had functional or clinical associations with cachexia and replicated in at least one study were entered into pathway analysis software to reveal possible network associations between genes. A total of 184 polymorphisms with functional or clinical relevance to cancer cachexia were identified in 92 candidate genes. Of these, 42 polymorphisms (in 33 genes) were replicated in more than one study with 13 polymorphisms found to influence two or more hallmarks of cachexia (i.e. inflammation, loss of fat mass and/or lean mass and reduced survival). Thirty-three genes were found to be significantly interconnected in two major networks with four genes (*ADIPOQ*, *IL6*, *NFKB1* and *TLR4*) interlinking both networks. Selection of candidate genes and polymorphisms is a key element of multigene study design. The present study provides an initial framework to select genes/polymorphisms for further study in cancer cachexia, and to develop their potential as susceptibility biomarkers of developing cachexia.

[Tan B. H. L., Ross J. A., Kaasa S., Skorpen F., Fearon K. C. H. and European Palliative Care Research Collaborative 2011 Identification of possible genetic polymorphisms involved in cancer cachexia: a systematic review. *J. Genet.* **90**, 165–177]

Introduction

Cancer cachexia is a multi-factorial syndrome characterized by chronic wasting involving loss of both adipose tissue and lean body mass (LBM). Depending on the tumour type, weight loss occurs in 30%–80% of the cancer patients and is severe (with loss of >10% of the initial body weight) in 15% (Dewys *et al.* 1980). Although certain tumour types are more commonly associated with cachexia, even with the same tumour type there are variations in the extent to which patients exhibit cachexia. Such variation maybe, in part, due to the patient's genotype rather than the tumour phenotype and/or tumour interaction. It is therefore likely there may be cachexia prone genotypes as well as cachexia resistant genotypes.

The cachexia syndrome is thought to result from a complex interplay of mechanisms involving the initiation of a host inflammatory response mediated by tumour-derived proinflammatory cytokines; the reprioritization of protein metabolism with induction of the acute phase response and mobilization of fat reserves; and activation of neuroendocrine pathways which may lead to hypermetabolism and increased catabolism (Tan *et al.* 2008). The wealth of known genetic variation in genes regulating the above mechanisms suggests their exploitable potential as biomarkers of inter-individual predictability of developing cachexia.

Single nucleotide polymorphisms (SNPs) are the most common type of stable genetic variations in the population

*For correspondence. E-mail: benjamin.tan@ed.ac.uk.

Keywords. human genetics; polymorphisms; cachexia.

(Brookes 1999). SNPs occur in approximately one in every thousand bases. There are several ways that SNPs can lead to an aberrant gene product. Promoter polymorphisms that alter DNA binding of transcription factors have the potential to decrease or increase gene expression; sequence variation in the 5′ untranslated region (UTR) could disrupt mRNA translation, and mutations in the 3′ UTR could affect mRNA cleavage, stability and export; finally, nonsynonymous SNPs in exons could alter protein function or activity. It has been estimated that 10% of all SNPs in the genome are functional, thereby having the potential of altering some biological process (Wjst 2004).

Over the past 10 years, considerable information has emerged on mechanisms of cancer cachexia of which inflammation is postulated to play a significant role. These mechanisms can be broadly grouped into five domains of interest: systemic inflammation, central energy balance, control of muscle metabolism/function, control of adipose tissue metabolism/function, and regulation of appetite.

This systematic literature review aims to explore genetic polymorphisms with known functional or clinical significance in potential candidate genes involved in the development of cancer cachexia within the above-mentioned domains. It also aims to identify the polymorphisms with the most likely potential as susceptibility markers for cancer cachexia.

Methods

The scientific literature published between 1986 and 2008 was searched in Medline and EmBase databases. In order to maximize the potential of identifying potential candidate genes, we utilized search terms that took into account the effects of cachexia. For example, 'survival' as a surrogate for accelerated death which may be due to cachexia. The term 'body composition' was used to identify potential genes of interest that may predispose individuals to higher/lower body mass which may influence the propensity to the development of cancer cachexia. 'Inflammation' was used as a main search term due to its postulated central role in the development of cancer cachexia. Overall, the following search terms were used to identify potential candidate genes/polymorphisms of interest: ((genes/genetics) or polymorphism(s)) and (inflammation or cancer or cachexia or weight loss or body composition or survival). The search was limited to studies on humans, in English language.

Following the initial retrieval of possible candidate genes, the genes were entered into a pathway functional analysis software (Ingenuity Pathways Analysis (IPA), Ingenuity Systems, Redwood, USA) to further identify related genes.

All identified candidate genes were then reviewed for functional polymorphisms or clinically significant polymorphism in terms of cachexia using OMIM and GeneRIF databases. Candidate genes were grouped into categories according to genes that regulate or code for the following:

- Inflammation
    - Innate immune receptors and mediators of the immune response
    - Cytokines
    - Cytokine receptors and related binding proteins
    - Acute phase protein reactants
- Central homeostasis
    - Energy production
    - Insulin like growth factors and related proteins
    - Corticosteroid signalling proteins
- Muscle
    - Muscle function and structure
    - Muscle proteolysis
- Adipose tissue
    - Adipogenesis
    - Lipid turnover and transport
    - Adipokines and adipokine receptors
- Appetite
- Others

Summary tables of polymorphisms are also 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. The summary tables also denote if a functional or clinical association with a polymorphism was replicated in more than one study. In addition, polymorphism reference numbers (rs numbers) were also recorded if known, as well as the minor allele frequency of the polymorphism based on a population with European ancestry derived from the HapMap or dbSNP databases.

Pathway analysis

It is likely that single genes are not adequate by themselves to affect disease risk; however, multiple genes within a single pathway may affect function enough to increase risk. To provide a more comprehensive assessment in terms of pathway involvement in cancer cachexia, we performed pathway-based analyses using the ingenuity pathway analysis (IPA) software. Based on the systematic literature review, the genes with variants that had functional or clinical associations replicated in at least one study were entered into the IPA analysis tool. These genes were termed focus genes. The IPA software was used to measure associations of these genes with other molecules, their network interactions, and biologic 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).

Results

A total of 184 polymorphisms with functional and/or clinical significance in terms of cachexia were identified in 92 genes.

Inflammation

Inflammation has long been associated with cancer cachexia. Systemic inflammation could result from tumour cell or host-cell-mediated production (Stewart *et al.* 2006). In experimental models, pro-inflammatory cytokines such as interleukin 1-beta (IL-1β), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα), and interferon gamma (IFNγ) may lead to an acute phase response and trigger tissue catabolism (Argiles *et al.* 2003).

Innate immune receptors and mediators of the immune response

Table 1 of electronic supplementary material at http://www.ias.ernet/jgenet/ explores the possible genetic determinants in the generation or suppression of the inflammatory response and how they may relate to cancer cachexia. These include variants in gene coding for the Toll-like receptor (TLR) family, and associated genes, which play an instructive role in innate immune responses as well as the subsequent induction of adaptive immune responses. TLRs are involved in triggering intra-cellular signals, culminating in the activation of nuclear factor (NF)-κB, where it participates in enhancing expression of other immuneregulatory substances (Kawai and Akira 2006). NF-κB is a transcriptional regulator that plays a central part in responses to inflammatory signalling. Polymorphisms in genes encoding for NF-κB and genes involved in the activation or inhibition of NF-κB are also shown in table 1 of the electronic supplementary material. Also of interest are variants in genes coding for cell adhesion molecules (CAMs) that are proteins located on the cell surface involved in binding with the other cells or with the extracellular matrix. CAMs are known to mediate migration of cells to sites of inflammation. Functional polymorphisms in the heat-shock proteins genes HSPA1L and HSPA1B have been noted in relation to inflammation and these are also displayed in table 1 of electronic supplementary material.

Cytokines and cytokine receptors

Cytokines are secreted proteins that play a role in the induction and effector phases of all immune and inflammatory responses. They serve diverse functions including induction of cell proliferation, mediating intercellular communication, chemotaxis and cell killing. Genetic variants of genes encoding pro-inflammatory and anti-inflammatory cytokines are presented in table 2 of electronic supplementary material.

Cytokines exert their effects via matching cell-surface receptors. Subsequent cascades of intracellular signalling then alter cell functions. This may include the upregulation and/or downregulation of several genes and their transcription factors, resulting in the production of other cytokines, an increase in the number of surface receptors for other molecules, or the suppression of their own effect by feedback inhibition (Ihle 1995). Table 3 of electronic supplementary material summarizes the variations in genes encoding cytokine receptors and related binding proteins.

Acute phase protein reactants

Acute-phase protein reactants (APPR) are proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) in response to inflammation (Stephens *et al.* 2008), and are also predictors of adverse outcomes in cancer patients. Some APPR also have roles in modulating the immune response such as C-reactive protein (CRP). Variants in genes coding for APPR are shown in table 4 of electronic supplementary material.

Central homeostasis

Body mass is controlled by the balance of energy intake and expenditure, like all thermodynamic systems. 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 below. The following section also explores genes involved in growth and development, and metabolic pathways common to both muscle and adipose tissues.

Energy production

Uncoupling proteins (UCPs) are transporters, present in the mitochondrial inner membrane, that mediate a regulated discharge of the proton gradient that is generated by the respiratory chain. This energy-dissipatory mechanism can serve functions such as thermogenesis, maintenance of the redox balance, or reduction in the production of reactive oxygen species (Ledesma *et al.* 2002). There are a total of five UCP homologs in humans. There are known significant polymorphisms within three UCP genes that may have a role in the development of cachexia and these are presented in table 5 of electronic supplementary material.

A polymorphism in the gene coding for triose phosphate isomerase (TPI) which plays an important role in glycolysis and is essential for efficient energy production is also shown in table 5 of electronic supplementary material. TPI deficiency leads to a metabolic block of the glycolytic pathway and hence a generalized impairment of cellular energy supply causing generalized skeletal muscle deficiency.

Insulin-like growth factors and related proteins

Insulin-like growth factors (IGFs) are polypeptides with high sequence similarity to insulin. IGFs are part of a complex system that cells use to communicate with their physiologic environment. This complex system (often referred to as the IGF 'axis') consists of two ligands (IGF-1 and IGF-2), two cell-surface receptors (IGF1R and IGF2R), and a family of six high-affinity IGF-binding proteins (IGFBP1–IGFBP6) (Jones and Clemmons 1995). This system regulates normal cellular metabolism, proliferation, differentiation and protecting against apoptotic signals (Jerome *et al.* 2003). High levels of IGF-1 and IGFBP3 have been implicated with poorer prognosis in certain types of cancers. Polymorphisms in genes coding for components of the IGF axis have been shown to affect serum levels of their respective proteins as well as body composition (see table 6 in electronic supplementary material).

Also shown in table 6 of electronic supplementary material are polymorphisms in the iodothyronine deiodinase, type 1 gene (DIO1) which activates thyroid hormone by converting the prohormone thyroxine (T4) by outer ring deiodination to bioactive triiodothyronine (T3). Thyroid hormone is known to interact with the GH-IGF-1 axis although the exact mechanism is unknown (Peeters *et al.* 2005).

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. The genetic variants of the components in the mechanism of corticosteroid signalling are examined in table 7 of electronic supplementary material.

Muscle

Muscle atrophy is known to occur in cancer cachexia. This results from the depression of muscle protein synthesis, an increase in muscle protein degradation, or a combination of both (Eley and Tisdale 2007). Experimental studies have also shown a shift in the myosin isoform content of skeletal muscle in cancer cachexia from type I to type II (Diffee *et al.* 2002). The following section examines the genetic variations that affect the structure and function of muscle as well as those that regulate muscle proteolysis.

Muscle structure and function

Genes involved in regulating muscle structure and function include those coding for ACTN3 (Alpha-actinin 3), which bind to actin at the Z-line within muscle fibres and act to anchor actin filaments, and IL15 (interleukin-15). 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). Polymorphisms in ACTN3, IL15 and IL15RA are shown in table 8 of electronic supplementary material.

Steroid androgens play an important role in determining LBM and muscle strength. Variants in the gene coding for the androgen receptor (AR), which is activated by binding of either androgenic hormones testosterone or dihydrotestosterone and are known to be capable of activating myogenic genes (Vlahopoulos *et al.* 2005), are also displayed in table 8 of electronic supplementary material. Polymorphisms associated with alterations fat free mass in the gene encoding the vitamin D receptor (VDR) are also included in table 8 of electronic supplementary material.

Muscle proteolysis

Angiotensin converting enzyme (ACE) plays a critical role in the renin-angiotensin system by catalysing the conversion of the inactive angiotensin I to angiotensin II, which is the physiologically active form of the hormone. 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.* 1996, 2001).

In atrophying muscles, the ubiquitin ligase, atrogin-1, is induced and this response is necessary for rapid atrophy. FOXO3 is known to act on atrogin-1 promoter to cause atrogin-1 transcription and this leads to dramatic atrophy of myotubes and muscle fibres (Sandri *et al.* 2004). Polymorphisms in *ACE* and *FOXO3A* genes are presented in table 9 of electronic supplementary material.

Adipose tissue

Body-fat depletion is a major component of weight loss in cancer cachexia. 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. There are three subtypes of the receptor (PPARα, PPARβ and PPARγ). PPARα is most commonly expressed in organs

and tissues in which fatty acid oxidation is active. 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 by orchestrating differentiation of new adipocytes and by inducing expression of genes promoting uptake of fatty acids, triglyceride synthesis and insulin sensitivity (Lehrke and Lazar 2005). Polymophisms in the *PPARA* and *PPARG* genes are shown in table 10 of electronic supplementary material.

Also of interest are the lipin proteins (lipin-1, lipin-2 and lipin-3) which are thought to be required for glycerolipid biosynthesis. They also act as transcriptional coactivators that regulate expression of lipid metabolism genes (Reue 2009). Variants of genes coding of lipin-1 (LPIN1) and lipin-2 (LPIN2) are also presented in table 10 of electronic supplementary material.

Lipid turnover and transport

During lipolysis, trigylcerides are broken down in a stepwise fashion to free fatty acids (FFAs). This process is partly modulated by the sympathetic nervous system-induced secretion of cathecholamines which act through β1, β2 and β3 adrenergic receptors (β-ARs) (Large *et al.* 2004). Signals between β-adrenergic receptors and effector proteins are integrated by G proteins which are composed of alpha, beta, and gamma subunits. Polymorphisms of genes coding for β-ARs and the beta subunits of the G proteins (GNB3) are shown in table 11 of electronic supplementary material.

Lipoprotein lipase (LPL) is an enzyme that hydrolyses lipids in lipoproteins and plays a central role in the overall lipid metabolism and transport (Mead *et al.* 2002). Fatty acid binding proteins (FABPs) are a family of carrier proteins for fatty acids and other lipophilic substances. These proteins are thought to facilitate the transfer of fatty acids between extracellular and intra-cellular membranes (Chmurzynska 2006). Polymorphisms in the *LPL* and *FABP* genes are shown in table 11 of electronic supplementary material.

Adiopkines and adipokine receptors

Adipose tissue is also recognized as a major endocrine organ, because the tissue synthesizes and secretes an array of protein hormones and signals (Fantuzzi 2005). 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 have an important role in the pathogenesis of cancer cachexia (Kerem *et al.* 2008).

Resistin is an adipocyte-derived proinflammatory cytokine. Resistin also appears to have effects on substrate metabolism through impairment of insulin action and insulin independent pathways (McTernan *et al.* 2006). There are three polymorphisms within the *RETN* gene which codes for resistin that may influence the development of cachexia (see table 12 in electronic supplementary material).

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). Adiponectin binds to a number of receptors including adiponectin receptors 1 and 2. An increase in adiponectin concentration has been assoicated with cachexia in patients with heart failure (McEntegart *et al.* 2007). Leptin is a protein hormone secreted by adipose tissue that 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 12 of electronic supplementary material.

Appetite regulation

Anorexia, defined as the loss of desire to eat, is common in patients with cancer cachexia. In addition to any effects of the tumour on the gastrointestinal tract and psychological depression, patients with cancer frequently have a decreased taste and smell of food (DeWys and Walters 1975). Cancer anorexia may be a result of an imbalance between orexigenic signals and anorexigenic signals. The following section explores the variants in the genes encoding these signals.

Ghrelin is synthesized principally in the stomach and is released in response to acute and chronic changes in nutritional state. In addition to having a powerful effect on the secretion of growth hormone, ghrelin stimulates food intake and transduces signals to hypothalamic regulatory nuclei that control energy homeostasis (Hosoda *et al.* 2002). The melanocortin 4 receptor, a G-protein coupled receptor, binds α-melanocyte stimulating hormone (α-MSH). Melanocortin 4 receptors have been found to be involved in feeding behaviour and the regulation of metabolism (Fan *et al.* 1997). Endocannabinoids, acting at brain cannabinoid type 1 receptors, play a role in stimulating appetite and ingestive behaviours, partly through interactions with more established orexigenic and anorexigenic signals (Kirkham 2005). Ciliary neurotrophic factor (CNTF) has been shown to activate hypothalamic leptin-like pathways which suppress food intake without triggering hunger signals or associated stress responses that are otherwise associated with food deprivation (Lambert *et al.* 2001). CNTF acts via CNTF receptors (CNTFRs) which are located in hypothalamic nuclei involved in feeding. Polymorphisms of interest in the genes encoding ghrelin (GHRL), melanocortin 4 receptor (MC4R), cannabinoid type 1 receptor (CNR1) and the CNTFR are given in table 13 of electronic supplementary material.

Others

Metallothionein (MT) is a family of cysteine-rich, low molecular weight proteins. MTs are encoded by a family

of genes consisting of 10 functional MT isoforms, and the encoded proteins are conventionally subdivided into four groups: MT-1, MT-2, MT-3 and MT-4 proteins. The physiological roles of MTs are not well understood but they are thought to play a role in the control of oxidative stress and protection against inflammation (Simpkins 2000).

The 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).

Glutamine : fructose-6-phosphate aminotransferase (GFAT) is the rate-limiting enzyme of the hexosamine biosynthetic pathway (HBP), which catalyses the conversion of fructose-6-phosphate to glucosamine-6-phosphate. The flux through the HBP has been shown to be linked to the regulation of energy intake and energy expenditure (Obici *et al.* 2002).

Fat mass and obesity associated (FTO) is thought to play a role in energy homeostasis but its exact function is unknown.

Genetic variants encoding the proteins discussed above may play a role in the development of cachexia and are listed in table 15 of electronic supplementary material.

Analysis of results

Out of 184 polymorphisms that have been identified, the functional or clinical significance of only 42 polymorphisms have been verified in more than one study.

Of these 42 polymorphisms, 13 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 13 polymorphisms represent the most promising candidates in terms of susceptibility biomarkers of cancer cachexia (table 1) and are explored in more detail below.

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)

TNF-α is a cytokine involved in systemic inflammation and is a member of a group of cytokines that stimulate the acute phase reaction. Within the *TNF* gene, the –308A allele (rs1800629) has been associated with an increased TNF-α production as well as a 6-fold increase in transcription of the *TNF* gene (Wilson *et al.* 1997; Sallakci *et al.* 2005). Interestingly, the A/A genotype has been linked to increased fat accumulation in women (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). Of note, obese individuals express 2.5-fold more TNF mRNA in fat tissue (Hotamisligil *et al.* 1995).

LTA, a member of the tumour necrosis factor family, is a cytokine produced by lymphocytes, and mediates a large variety of inflammatory and immunostimulatory, responses. 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).

IL-1β is a cytokine protein which is encoded by the *IL1B* gene and is an important mediator of the 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). The -31C and -511T alleles are linked with poorer progression-free survival and overall survival in advanced gastric cancer (Graziano *et al.* 2005). Increased IL-1β levels have been linked to a synonymous C to T polymorphism at nucleotide position 3953 (rs1143634) (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).

IL-6 is a cytokine involved in a wide variety of biological functions. It is critical for 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 (Berthier *et al.* 2003; Roth *et al.* 2003).

IGF-1 plays an important role in childhood growth and continues to have anabolic effects in adults. IGF-1 is one of the most potent natural activators of the Akt signalling pathway, a stimulator of cell growth and multiplication. IGF-1 also mediates many of the growth-promoting effects of growth hormone (GH) (Jones and Clemmons 1995). A CA repeat polymorphism is found within the promoter region of the *IGF1* gene. The 19CA repeat allele is associated with both lower serum IGF-I levels and IGFBP-3 levels (Rosen *et al.* 1998; Morimoto *et al.* 2005). The 19CA repeat allele is also associated with reduced risk of weight gain (Landmann *et al.* 2006; Voorhoeve *et al.* 2006). Of note, increasing IGF-1 levels have been associated with poorer prognosis in oesophageal cancer patients (Sohda *et al.* 2004).

Table 1. Polymorphisms replicated in more than one study and with at least two effects on clinical features associated with cancer cachexia ($n = 13$).

Gene	Polymorphism	Minor allele frequency	Functional significance	Systemic inflammation	BMI/fat mass	Lean mass /strength	Cancer survival
SELP	A37674C (715 Thr→Pro) [rs6136]	9%	Decreased serum P-selectin levels (Miller *et al.* 2004; Volcik *et al.* 2006)	↓			↑
TNF	G-308A [rs1800629]	17%	Increased *TNF-α* production (Sallakci *et al.* 2005) Six-fold increase in transcription of *TNF-α* (Wilson *et al.* 1997)	↑	↑		
	C-863A [rs1800630]	15%	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)	↓	↓		
LTA	A252G (Intron 1) [rs909253]	36%	Increased serum *TNF-α* levels (Stuber *et al.* 1996; McArthur *et al.* 2002)	↑			↓
IL-1β	C-31T [rs1143627]	36%	Increased expression of *IL-1β* gene with T allele (Lind *et al.* 2007) 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)	↑			↑
	C-511T [rs16944]	36%	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)	↓			↓
IL-1β	C3953T (3954) (synonymous) [rs1143634]	21%	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)	↑	↓		↓
IL6	G-174C [rs1800795]	46%	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)	↓	↑	↓	
IGF1	CA repeat promoter polymorphism 19 CA repeats (192bp)	?	Lower serum IGF-I levels (Rosen *et al.* 1998; Morimoto *et al.* 2005) Lower IGFBP-3 levels (Morimoto *et al.* 2005)		↓		↑
ACE	Insertion/deletion (I/D) polymorphism Intron 16 (287bp) [rs4646994]	?	I allele associated with lower ACE levels (Tiret *et al.* 1992)		↑	↑	↑
LPL	447 Ser→TER [rs328]	12%	Significantly lower IL-8 levels (Ak *et al.* 2007) Increased LPL activity (Kozaki *et al.* 1993; Groenemeijer *et al.* 1997)	↓	↓		
RETN	C-420G [rs1862513]	35%	Increased plasma resistin (Cho *et al.* 2004; Osawa *et al.* 2007)	↑	↑		
ADIPOQ	T45G (synonymous) [rs2241766]		Increased plasma adiponectin (Berthier *et al.* 2005; Mackevics *et al.* 2006)		↓	↑	

↑ Increase; ↓ decrease; ?, allele frequency unknown.

Angiotensin converting enzyme (ACE) plays a critical role in the renin-angiotensin system by catalysing the conversion of the inactive angiotensin I to angiotensin II, which is the physiologically active form of the hormone. 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.* 1996, 2001). A common insertion/deletion (I/D) polymorphism (rs4646994) is present in intron 16 of the *ACE* gene. The I allele is associated with lower circulating ACE levels (Tiret *et al.* 1992). The D allele is associated with increased strength gains thorough isometric training (Folland *et al.* 2000) and also associated with obesity (El-Hazmi and Warsy 2003; Riera-Fortuny *et al.* 2005). The D/D genotype is linked with increased survival in women with colorectal cancer (Rocken *et al.* 2007).

Lipoprotein lipase (LPL) is an enzyme that hydrolysis lipids in lipoproteins and plays a central role in the overall lipid metabolism and transport (Mead *et al.* 2002). The rs328 polymorphism in the *LPL* gene results in 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).

Resistin is an adipocyte-derived pro-inflammatory cytokine. 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 to 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).

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). An increase in adiponectin concentration is associated with cachexia in patients with heart failure (McEntegart *et al.* 2007). The *ADIPOQ* gene, which codes for 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).

Pathway analysis

The 42 polymorphisms which have been verified in more than one study were found across 33 genes. These genes were entered into the IPA algorithm as focus genes and were found to be significantly interconnected in two major networks (table 2). Using the 'overlapping network' feature of IPA, we found that these networks were joined together by few genes, namely *ADIPOQ, IL6, NFKB1 and TLR4*. The two networks are presented in figures 1 and 2.

Discussion

Support for a heritable component to cachexia has recently been highlighted in several studies. The IL6 -634G allele is associated with increased susceptibility to cachexia and decreased survival time of Chinese patients suffering from pancreatic cancer (Zhang *et al.* 2008). Another study by

Table 2. Ingenuity pathway analysis of genes that were replicated in more than one study ($n = 33$).

Network	Molecules*	Calculated score	Focus genes	Top functions of network
1	**ADIPOQ**, Akt, Ap1, **CCL2**, ERK, ERK1/2, Gm-csf, histone h3, histone h4, IFN beta, **IFNG**, **IGF1**,**IGFBP3**, IgG, **IL6,** IL12 (complex), **IL1B**, immunoglobulin, interferon alpha, Jnk, LDL, **LEP**, Mapk,**NFKB1**, NFkB (complex), P38 MAPK, PDGF BB, PI3K, **PPARG**, **RETN**, Sod, **TLR2**,**TLR4**,**TNF**, Vegf	24	14	Connective tissue disorders, inflammatory disease, skeletal and muscular disorders
2	**ADIPOQ**, **ADRB2**, BIRC3, BTG2, CAMP, CREBBP, **CRP**, CSF1, F2, GSK3B, HDAC3, HGF, HMGB1 (includes EG:3146), HSPD1, IKBKB, **IL6**, IL17A (includes EG:3605), IL6R, IRS1, **LPL**, LTF, NFIC, **NFKB1**, NOS2, **NR3C1**, OSM, PLD1, PLG, RELA, **SELP**, SIRT1, **TLR4**, TNFSF12, TP53,TSC22D3	13	9	Skeletal and muscular system development and function, cell death, cellular movement

*Focus genes are in bold.

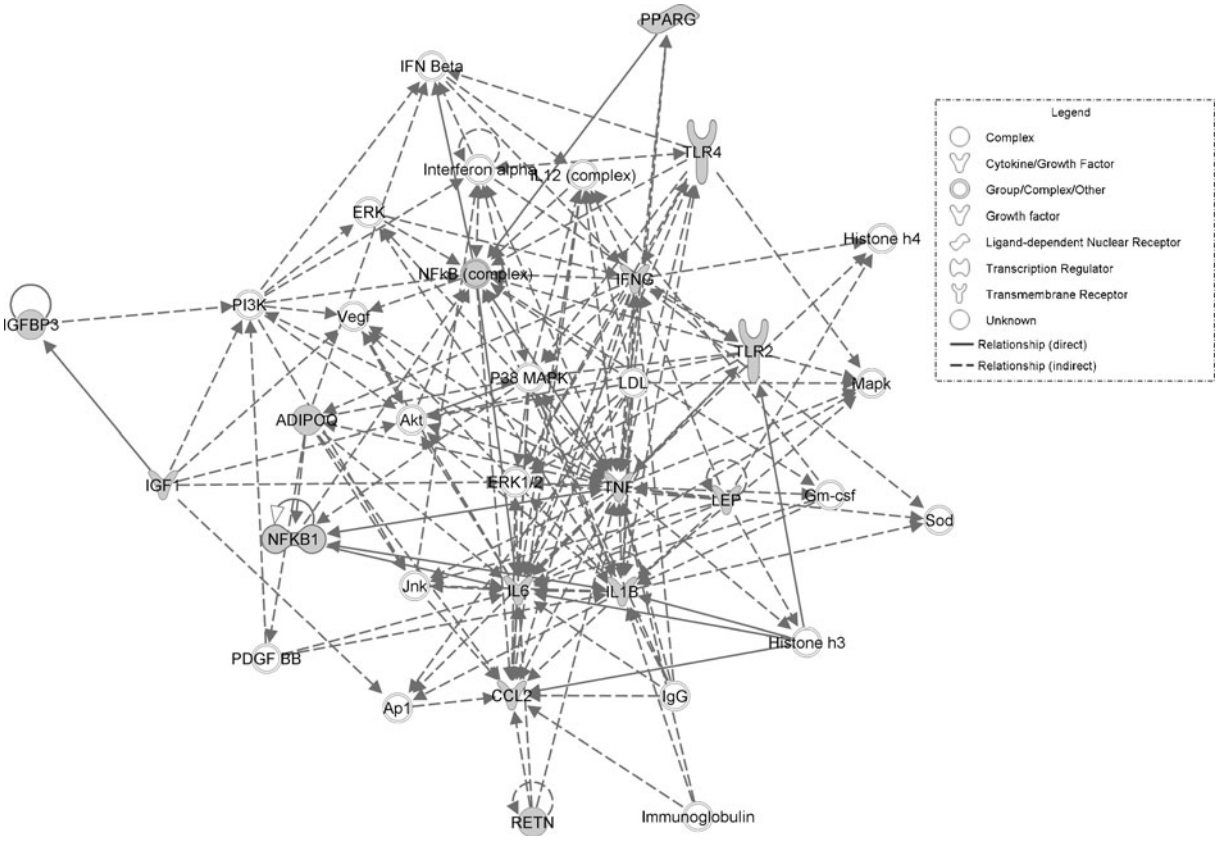

Figure 1. Connection map for first ranked network. Genes with variants that had functional or clinical associations replicated in at least one study were entered into the ingenuity pathway analysis software for an unsupervised functional analysis to discern regulatory networks that involved these molecules. 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.

Deans *et al.* (2009) revealed that the IL10 -1082GG genotype was associated with 2.3 times increased risk of developing cachexia in patients with gastroesophageal malignancy.

However, like many complex conditions and diseases, the risk of developing cancer cachexia is probably determined by multiple genetic factors and environmental factors are likely to add to the heterogeneity of the condition. Although the number of reports on polymorphic gene variants associated with multi-factorial diseases and conditions are dramatically growing, very few studies provide firm and reliable evidence of causative relationships between these polymorphisms and risk or pathogenesis. Indeed, in a recent review of allelic association with common disease phenotypes, only six of the 166 associations subjected to multiple evaluations were confirmed consistently (Hirschhorn *et al.* 2002). Possible causes of false-positive association studies include population stratification, variable linkage disequilibrium and genotype misclassification. In addition, in many of these studies, the possible effects of single gene variants were assessed in situations when combined impacts of multiple factors could be expected (Loktionov 2003). In the present study, we have identified 42 polymorphisms out of 184 with a potential role in the development of cachexia that have been independently verified in at least one repeat study.

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. In this study, 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.

Of the 42 polymorphisms with a potential role in the development of cachexia that have been independently verified in at least one repeat study, 13 polymorphisms have been shown to have more than one effect on clinical features associated with cancer cachexia. These 13 polymorphisms are likely to be the most promising candidates in terms of susceptibility biomarkers of cancer cachexia and should be further investigated.

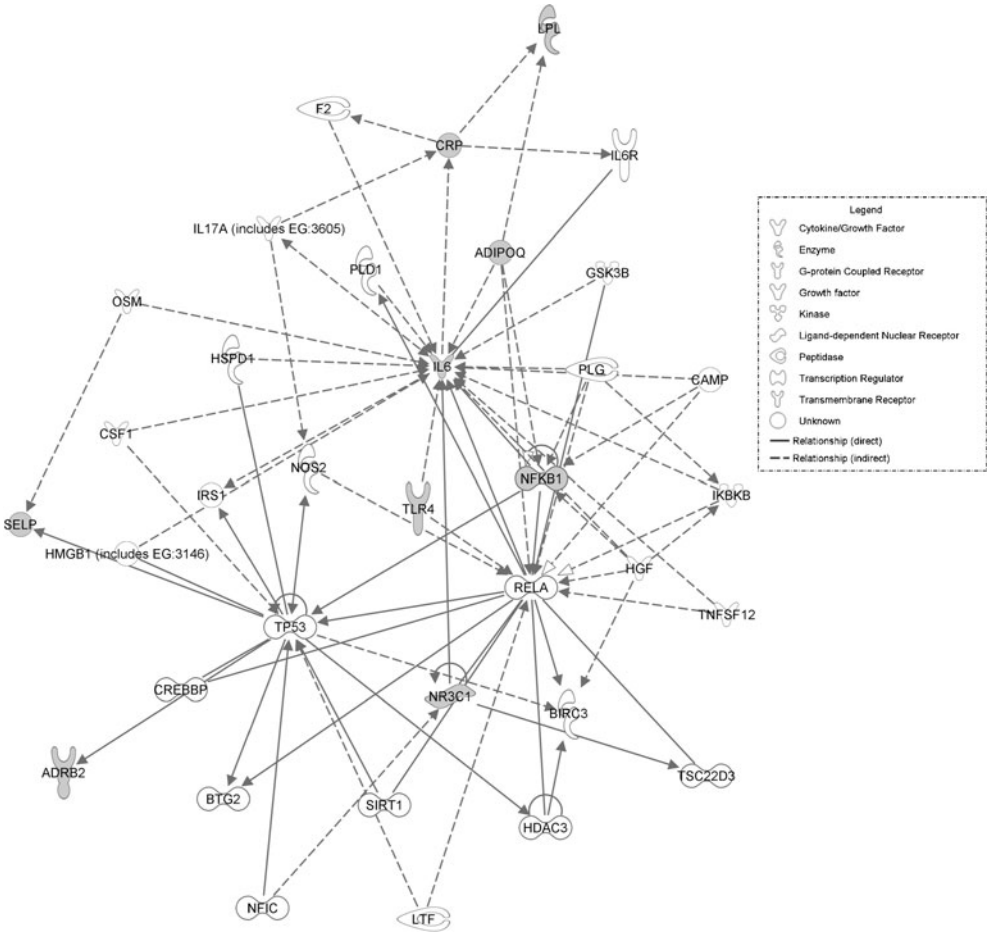

Figure 2. Connection map for second ranked network. Genes with variants that had functional or clinical associations replicated in at least one study were entered into the ingenuity pathway analysis software for an unsupervised functional analysis to discern regulatory networks that involved these molecules. 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.

Pathway analysis of the independently verified genes has revealed four genes interlinking two putative major networks involved in the development of cancer cachexia. These four genes (*ADIPOQ, IL6, NFKB1* and *TLR4*) may have central roles in the pathogenesis of cancer cachexia and should be further investigated.

It should also be emphasized that while certain variant combinations can predispose to a condition, others may be protective against the same condition. For example, polymorphisms resulting in an increase in systemic inflammation, a decrease in lean or fat mass and decreased survival in cancer are likely to be cachexia prone variants. On the other hand, polymorphisms that result in a decrease in systemic inflammation, an increase in body mass and improved survival in cancer are likely to be cachexia resistant variants.

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 of 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).

The present study has provided an initial framework to select and study the possible genetic variance in developing cancer cachexia by identifying polymorphisms with putative functional and/or clinical significance in relation to the development of cachexia. The identification of genetic variants that have undergone repeat studies allows the selection of robust and reliable candidates. Furthermore, prioritization of the most likely genetic variants that are likely to influence the development of cachexia have been derived by selecting polymorphisms that influence two or more hallmarks of cachexia (i.e. systemic inflammation, loss of fat mass, loss of lean mass and reduced survival).

Acknowledgements

The authors are part of the European Palliative Care Research Collaborative (EPCRC) and are supported by a European Union 6th Framework Grant (LSHC-CT-2006-037777).

References

Ak K., Isbir, S., Tekeli A., Ergen A., Atalan N., Dogan S. *et al*. 2007 Presence of lipoprotein lipase S447X stop codon affects the magnitude of interleukin 8 release after cardiac surgery with cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* **134**, 477–483.

Alexiou D., Karayiannakis A. J., Syrigos K. N., Zbar A., Kremmyda A., Bramis I. *et al.* 2001 Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur. J. Cancer* **37**, 2392–2397.

Alexiou D., Karayiannakis A. J., Syrigos K. N., Zbar A., Sekara E., Michail P. *et al.* 2003 Clinical significance of serum levels of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in gastric cancer patients. *Am. J. Gastroenterol.* **98**, 478–485.

Argiles J. M., Busquets S. and Lopez-Soriano F. J. 2003 Cytokines in the pathogenesis of cancer cachexia. *Curr. Opin. Clin. Nutr. Metab. Care* **6**, 401–406.

Awomoyi A. A., Charurat M., Marchant A., Miller E. N., Blackwell J. M., McAdam K. P. *et al.* 2005. Polymorphism in IL1B: IL1B-511 association with tuberculosis and decreased lipopolysaccharide-induced IL-1beta in IFN-gamma primed ex-vivo whole blood assay. *J. Endotoxin Res.* **11**, 281–286.

Barber M. D., Powell J. J., Lynch S. F., Fearon K. C. and Ross J. A. 2000 A polymorphism of the interleukin-1 beta gene influences survival in pancreatic cancer. *Br. J. Cancer* **83**, 1443–1447.

Berthier M. T., Paradis A. M., Tchernof A., Bergeron J., Prud'homme D., Despres J. P. *et al.* 2003 The interleukin 6–174G/C polymorphism is associated with indices of obesity in men. *J. Hum. Genet.* **48**, 14–19.

Berthier M. T., Houde A., Cote M., Paradis A. M., Mauriege P., Bergeron J. *et al*. 2005 Impact of adiponectin gene polymorphisms on plasma lipoprotein and adiponectin concentrations of viscerally obese men. *J. Lipid Res.* **46**, 237–244.

Brink M., Wellen J. and Delafontaine P. 1996. Angiotensin II causes weight loss and decreases circulating insulin-like growth factor I in rats through a pressor-independent mechanism. *J. Clin. Invest.* **97**, 2509–2516.

Brink M., Price S. R., Chrast J., Bailey J. L., Anwar A., Mitch W. E. *et al*. 2001 Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. *Endocrinology* **142**, 1489–1496.

Brookes A. J. 1999 The essence of SNPs. *Gene* **234**, 177–186.

Chmurzynska A. 2006 The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. *J. Appl. Genet.* **47**, 39–48.

Cho Y. M., Youn B. S., Chung S. S., Kim K. W., Lee H. K., Yu K. Y. *et al.* 2004. Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia* **47**, 559–565.

Day C. P., Grove J., Daly A. K., Stewart M. W., Avery P. J. and Walker M. 1998 Tumour necrosis factor-alpha gene promoter polymorphism and decreased insulin resistance. *Diabetologia* **41**, 430–434.

Deans D. A., Tan B. H., Ross J. A., Rose-Zerilli M., Wigmore S. J., Howell W. M. *et al.* 2009 Cancer cachexia is associated with the IL10 -1082 gene promoter polymorphism in patients with gastroesophageal malignancy. *Am. J. Clin. Nutr.* **89**, 1164–1172.

Degawa-Yamauchi M., Bovenkerk J. E., Juliar B. E., Watson W., Kerr K., Jones R. *et al*. 2003 Serum resistin (FIZZ3) protein is increased in obese humans. *J. Clin. Endocrinol. Metab.* **88**, 5452–5455.

DeWys W. D. and Walters K. 1975 Abnormalities of taste sensation in cancer patients. *Cancer* **36**, 1888–1896.

Dewys W. D., Begg C., Lavin P. T., Band P. R., Bennett J. M., Bertino J. R. *et al.* 1980 Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern cooperative oncology group. *Am. J. Med.* **69**, 491–497.

Diez J. J. and Iglesias P. 2003 The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur. J. Endocrinol.* **148**, 293–300.

Diffee G. M., Kalfas K., Al-Majid S. and McCarthy D. O. 2002 Altered expression of skeletal muscle myosin isoforms in cancer cachexia. *Am. J. Physiol. Cell Physiol.* **283**, C1376–C1382.

Eley H. L. and Tisdale M. J. 2007 Skeletal muscle atrophy, a link between depression of protein synthesis and increase in degradation. *J. Biol. Chem.* **282**, 7087–7097.

El-Hazmi M. A. and Warsy A. S. 2003 Increased frequency of angiotensin-converting enzyme DD genotype in Saudi overweight and obese patients. *Ann. Saudi Med.* **23**, 24–27.

Fan W., Boston B. A., Kesterson R. A., Hruby V. J. and Cone R. D. 1997 Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **385**, 165–168.

Fantuzzi G. 2005 Adipose tissue, adipokines, and inflammation. *J. Allergy Clin. Immunol.* **115**, 911–919.

Fishman D., Faulds G., Jeffery R., Mohamed-Ali V., Yudkin J. S., Humphries S. *et al*. 1998 The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J. Clin. Invest.* **102**, 1369–1376.

Folland J., Leach B., Little T., Hawker K., Myerson S., Montgomery H. *et al.* 2000 Angiotensin-converting enzyme genotype affects the response of human skeletal muscle to functional overload. *Exp. Physiol.* **85**, 575–579.

Fredrix E. W., Soeters P. B., Wouters E. F., Deerenberg I. M., von Meyenfeldt M. F. and Saris W. H. 1991 Effect of different tumor types on resting energy expenditure. *Cancer Res.* **51**, 6138–6141.

Graziano F., Ruzzo A., Santini D., Humar B., Tonini G., Catalano V. *et al.* 2005 Prognostic role of interleukin-1beta gene and interleukin-1 receptor antagonist gene polymorphisms in patients with advanced gastric cancer. *J. Clin. Oncol.* **23**, 2339–2345.

Groenemeijer B. E., Hallman M. D., Reymer P. W., Gagne E., Kuivenhoven J. A., Bruin T. *et al.* 1997 Genetic variant showing a positive interaction with beta-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. REGRESS study group. *Circulation* **95**, 2628–2635.

Hernandez-Guerrero C., Monzon-Bordonaba F., Jimenez-Zamudio L., Ahued-Ahued R., Arechavaleta-Velasco F., Strauss J. F. 3rd *et al.* 2003 In-vitro secretion of proinflammatory cytokines by human amniochorion carrying hyper-responsive gene polymorphisms of tumour necrosis factor-alpha and interleukin-1beta. *Mol. Hum. Reprod.* **9**, 625–629.

Hirschhorn J. N., Lohmueller K., Byrne E. and Hirschhorn K. 2002 A comprehensive review of genetic association studies. *Genet. Med.* **4**, 45–61.

Hoffstedt J., Eriksson P., Hellstrom L., Rossner S., Ryden M. and Arner P. 2000 Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. *Diabetologia* **43**, 117–120.

Hosoda H., Kojima M. and Kangawa K. 2002 Ghrelin and the regulation of food intake and energy balance. *Mol. Interv.* **2**, 494–503.

Hotamisligil G. S., Arner P., Caro J. F., Atkinson R. L. and Spiegelman B. M. 1995 Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J. Clin. Invest.* **95**, 2409–2415.

Huang A. Q., Hu Y. H., Zhan S. Y., Xu B., Pang Z. C., Cao W. H. *et al.* 2006 Lipoprotein lipase gene S447X polymorphism modulates the relation between central obesity and serum lipids, a twin study. *Int. J. Obes.* **30**, 1693–1701.

Ihle J. N. 1995 Cytokine receptor signalling. *Nature* **377**, 591–594.

Jerome L., Shiry L. and Leyland-Jones B. 2003 Deregulation of the IGF axis in cancer: epidemiological evidence and potential therapeutic interventions. *Endocr. Relat. Cancer* **10**, 561–578.

Jones J. I. and Clemmons D. R. 1995 Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* **16**, 3–34.

Kaluza W., Reuss E., Grossmann S., Hug R., Schopf R. E., Galle P. R. *et al*. 2000 Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. *J. Invest. Dermatol.* **114**, 1180–1183.

Kawai T. and Akira S. 2006 TLR signaling. *Cell Death Differ.* **13**, 816–825.

Kerem M., Ferahkose Z., Yilmaz U. T., Pasaoglu H., Ofluoglu E., Bedirli A. *et al.* 2008 Adipokines and ghrelin in gastric cancer cachexia. *World J. Gastroenterol.* **14**, 3633–3641.

Kirkham T. C. 2005 Endocannabinoids in the regulation of appetite and body weight. *Behav. Pharmacol.* **16**, 297–313.

Kishimoto T. 2005 Interleukin-6: from basic science to medicine-40 years in immunology. *Annu. Rev. Immunol.* **23**, 1–21.

Kozaki K., Gotoda T., Kawamura M., Shimano H., Yazaki Y., Ouchi Y. *et al.* 1993 Mutational analysis of human lipoprotein lipase by carboxy-terminal truncation. *J. Lipid Res.* **34**, 1765–1772.

Kusminski C. M., da Silva N. F., Creely S. J., Fisher F. M., Harte A. L., Baker A. R. *et al.* 2007 The in vitro effects of resistin on the innate immune signaling pathway in isolated human subcutaneous adipocytes. *J. Clin. Endocrinol. Metab.* **92**, 270–276.

Lambert P. D., Anderson K. D., Sleeman M. W., Wong V., Tan J., Hijarunguru A. *et al.* 2001 Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. *Proc. Natl. Acad. Sci. USA* **98**, 4652–4657.

Landmann E., Geller F., Schilling J., Rudloff S., Foeller-Gaudier E. and Gortner L. 2006 Absence of the wild-type allele (192 base pairs) of a polymorphism in the promoter region of the IGF-I gene but not a polymorphism in the insulin gene variable number of tandem repeat locus is associated with accelerated weight gain in infancy. *Pediatrics* **118**, 2374–2379.

Large V., Peroni O., Letexier D., Ray H. and Beylot M. 2004 Metabolism of lipids in human white adipocyte. *Diabetes Metab.* **30**, 294–309.

Ledesma A., de Lacoba M. G. and Rial E. 2002 The mitochondrial uncoupling proteins. *Genome Biol.* **3**, 3015.1–3015.9.

Legaspi A., Jeevanandam M., Starnes Jr H. F. and Brennan M. F. 1987 Whole body lipid and energy metabolism in the cancer patient. *Metabolism* **36**, 958–963.

Lehrke M. and Lazar M. A. 2005 The many faces of PPARgamma. *Cell* **123**, 993–999.

Lind H., Haugen A. and Zienolddiny S. 2007 Differential binding of proteins to the IL1B -31 T/C polymorphism in lung epithelial cells. *Cytokine* **38**, 43–48.

Loktionov A. 2003 Common gene polymorphisms and nutrition: emerging links with pathogenesis of multifactorial chronic diseases (review). *J. Nutr. Biochem.* **14**, 426–451.

Loos R. J., Ruchat S., Rankinen T., Tremblay A., Perusse L. and Bouchard C. 2007 Adiponectin and adiponectin receptor gene variants in relation to resting metabolic rate, respiratory quotient, and adiposity-related phenotypes in the Quebec Family Study. *Am. J. Clin. Nutr.* **85**, 26–34.

Mackevics V., Heid I. M., Wagner S. A., Cip P., Doppelmayr H., Lejnieks A. *et al.* 2006 The adiponectin gene is associated with adiponectin levels but not with characteristics of the insulin resistance syndrome in healthy Caucasians. *Eur. J. Hum. Genet.* **14**, 349–356.

McArthur J. A., Zhang Q. and Quasney M. W. 2002 Association between the A/A genotype at the lymphotoxin-alpha + 250 site and increased mortality in children with positive blood cultures. *Pediatr. Crit. Care Med.* **3**, 341–344.

McEntegart M. B., Awede B., Petrie M. C., Sattar N., Dunn F. G., MacFarlane N. G. *et al.* 2007 Increase in serum adiponectin concentration in patients with heart failure and cachexia: relationship with leptin, other cytokines, and B-type natriuretic peptide. *Eur. Heart J.* **28**, 829–835.

McTernan P. G., Kusminski C. M. and Kumar S. 2006 Resistin. *Curr. Opin. Lipidol.* **17**, 170–175.

Mead J. R., Irvine S. A. and Ramji D. P. 2002 Lipoprotein lipase: structure, function, regulation, and role in disease. *J. Mol. Med.* **80**, 753–769.

Miller M. A., Kerry S. M., Dong Y., Strazzullo P. and Cappuccio F. P. 2004 Association between the Thr715Pro P-selectin gene polymorphism and soluble P-selectin levels in a multiethnic population in South London. *Thromb. Haemost.* **92**, 1060–1065.

Morimoto L. M., Newcomb P. A., White E., Bigler J. and Potter J. D. 2005 Variation in plasma insulin-like growth factor-1 and insulin-like growth factor binding protein-3: genetic factors. *Cancer Epidemiol. Biomarkers Prev.* **14**, 1394–1401.

Nagaev I., Bokarewa M., Tarkowski A. and Smith U. 2006 Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adipocytes. *PLoS One* **1**, e31.

Norata G. D., Ongari M., Garlaschelli K., Tibolla G., Grigore L., Raselli S. *et al.* 2007 Effect of the −420C/G variant of the resistin gene promoter on metabolic syndrome, obesity, myocardial infarction and kidney dysfunction. *J. Intern. Med.* **262**, 104–112.

Obici S., Wang J., Chowdury R., Feng Z., Siddhanta U., Morgan K. *et al.* 2002 Identification of a biochemical link between energy intake and energy expenditure. *J. Clin. Invest.* **109**, 1599–1605.

Osawa H., Tabara Y., Kawamoto R., Ohashi J., Ochi M., Onuma H. *et al.* 2007 Plasma resistin, associated with single nucleotide polymorphism -420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. *Diabetes Care* **30**, 1501–1506.

Peeters R. P., van den Beld A. W., van Toor H., Uitterlinden A. G., Janssen J. A., Lamberts S. W. *et al.* 2005 A polymorphism in type I deiodinase is associated with circulating free insulin-like growth factor I levels and body composition in humans. *J. Clin. Endocrinol. Metab.* **90**, 256–263.

Quinn L. S., Anderson B. G., Drivdahl R. H., Alvarez B. and Argiles J. M. 2002 Overexpression of interleukin-15 induces skeletal muscle hypertrophy in vitro: implications for treatment of muscle wasting disorders. *Exp. Cell Res.* **280**, 55–63.

Reue K. 2009 The lipin family: mutations and metabolism. *Curr. Opin. Lipidol.* **20**, 165–170.

Riera-Fortuny C., Real J. T., Chaves F. J., Morales-Suarez-Varela M., Martinez-Triguero M. L., Morillas-Arino C. *et al.* 2005 The relation between obesity, abdominal fat deposit and the

angiotensin-converting enzyme gene I/D polymorphism and its association with coronary heart disease. *Int. J. Obes.* **29**, 78–84.

Rocken C., Neumann K., Carl-McGrath S., Lage H., Ebert M. P., Dierkes J. *et al.* 2007 The gene polymorphism of the angiotensin I-converting enzyme correlates with tumor size and patient survival in colorectal cancer patients. *Neoplasia* **9**, 716–722.

Rosen C. J., Kurland E. S., Vereault D., Adler R. A., Rackoff P. J., Craig W. Y. *et al.* 1998 Association between serum insulin growth factor-I (IGF-I) and a simple sequence repeat in IGF-I gene: implications for genetic studies of bone mineral density. *J. Clin. Endocrinol. Metab.* **83**, 2286–2290.

Roth S. M., Schrager M. A., Lee M. R., Metter E. J., Hurley B. F. and Ferrell R. E. 2003 Interleukin-6 (IL6) genotype is associated with fat-free mass in men but not women. *J. Gerontol. (A Biol. Sci. Med. Sci.)* **58**, B1085–B1088.

Sallakci N., Akcurin G., Koksoy S., Kardelen F., Uguz A., Coskun M. *et al.* 2005 TNF-alpha G-308A polymorphism is associated with rheumatic fever and correlates with increased TNF-alpha production. *J. Autoimmun.* **25**, 150–154.

Sandri M., Sandri C., Gilbert A., Skurk C., Calabria E., Picard A. *et al.* 2004 Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. *Cell* **117**, 399–412.

Sharma S., Sharma A., Kumar S., Sharma S. K. and Ghosh B. 2006 Association of TNF haplotypes with asthma, serum IgE levels, and correlation with serum TNF-alpha levels. *Am. J. Respir. Cell Mol. Biol.* **35**, 488–495.

Shimura T., Hagihara M., Takebe K., Munkhbat B., Odaka T., Kato H. *et al.* 1994 The study of tumor necrosis factor beta gene polymorphism in lung cancer patients. *Cancer* **73**, 1184–1188.

Shimura T., Hagihara M., Takebe K., Munkhbat B., Ogoshi K., Mitomi T. *et al.* 1995 10.5-kb homozygote of tumor necrosis factor-beta gene is associated with a better prognosis in gastric cancer patients. *Cancer* **75**, 1450–1453.

Silswal N., Singh A. K., Aruna B., Mukhopadhyay S., Ghosh S. and Ehtesham N. Z. 2005 Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. *Biochem. Biophys. Res. Commun.* **334**, 1092–1101.

Simpkins C. O. 2000 Metallothionein in human disease. *Cell Mol. Biol.* **46**, 465–488.

Skoog T., van't Hooft F. M., Kallin B., Jovinge S., Boquist S., Nilsson J. *et al.* 1999 A common functional polymorphism (C–>A substitution at position -863) in the promoter region of the tumour necrosis factor-alpha (TNF-alpha) gene associated with reduced circulating levels of TNF-alpha. *Hum. Mol. Genet.* **8**, 1443–1449.

Sohda M., Kato H., Miyazaki T., Nakajima M., Fukuchi M., Manda R. *et al.* 2004 The role of insulin-like growth factor 1 and insulin-like growth factor binding protein 3 in human esophageal cancer. *Anticancer Res.* **24**, 3029–3034.

Stephens N. A., Skipworth R. J. and Fearon K. C. 2008 Cachexia, survival and the acute phase response. *Curr. Opin. Support Palliat. Care* **2**, 267–274.

Stewart G. D., Skipworth R. J. and Fearon K. C. 2006 Cancer cachexia and fatigue. *Clin. Med.* **6**, 140–143.

Strandberg L., Lorentzon M., Hellqvist A., Nilsson S., Wallenius V., Ohlsson C. *et al.* 2006 Interleukin-1 system gene polymorphisms are associated with fat mass in young men. *J. Clin. Endocrinol. Metab.* **91**, 2749–2754.

Stuber F., Petersen M., Bokelmann F. and Schade U. 1996 A genomic polymorphism within the tumor necrosis factor locus influences plasma tumor necrosis factor-alpha concentrations and outcome of patients with severe sepsis. *Crit. Care Med.* **24**, 381–384.

Tabor H. K., Risch N. J. and Myers R. M. 2002 Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat. Rev. Genet.* **3**, 391–397.

Tan B. H., Deans D. A., Skipworth R. J., Ross J. A. and Fearon K. C. 2008 Biomarkers for cancer cachexia: is there also a genetic component to cachexia? *Support Care Cancer* **16**, 229–234.

Tiret L., Rigat B., Visvikis S., Breda C., Corvol P., Cambien F. *et al.* 1992 Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am. J. Hum. Genet.* **51**, 197–205.

Tolusso B., Pietrapertosa D., Morelli A., De Santis M., Gremese E., Farina G. *et al.* 2006 IL-1B and IL-1RN gene polymorphisms in rheumatoid arthritis: relationship with protein plasma levels and response to therapy. *Pharmacogenomics* **7**, 683–695.

Torra I. P., Chinetti G., Duval C., Fruchart J. C. and Staels B. 2001 Peroxisome proliferator-activated receptors: from transcriptional control to clinical practice. *Curr. Opin. Lipidol.* **12**, 245–254.

Vlahopoulos S., Zimmer W. E., Jenster G., Belaguli N. S., Balk S. P., Brinkmann A. O. *et al.* 2005 Recruitment of the androgen receptor via serum response factor facilitates expression of a myogenic gene. *J. Biol. Chem.* **280**, 7786–7792.

Volcik K. A., Ballantyne C. M., Coresh J., Folsom A. R., Wu K. K. and Boerwinkle E. 2006 P-selectin Thr715Pro polymorphism predicts P-selectin levels but not risk of incident coronary heart disease or ischemic stroke in a cohort of 14595 participants: the Atherosclerosis Risk in Communities Study. *Atherosclerosis* **186**, 74–79.

von Kugelgen I. 2006 Pharmacological profiles of cloned mammalian P2Y-receptor subtypes. *Pharmacol. Ther.* **110**, 415–432.

Voorhoeve P. G., van Rossum E. F., Te Velde S. J., Koper J. W., Kemper H. C., Lamberts S. W. *et al.* 2006 Association between an IGF-I gene polymorphism and body fatness: differences between generations. *Eur. J. Endocrinol.* **154**, 379–388.

Wen A. Q., Wang J., Feng K., Zhu P. F., Wang Z. G. and Jiang J. X. 2006 Effects of haplotypes in the interleukin 1beta promoter on lipopolysaccharide-induced interleukin 1beta expression. *Shock* **26**, 25–30.

Wilson A. G., Symons J. A., McDowell T. L., McDevitt H. O. and Duff G. W. 1997 Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc. Natl. Acad. Sci. USA* **94**, 3195–3199.

Wjst M. 2004 Target SNP selection in complex disease association studies. *BMC Bioinformatics* **5**, 92.

Yang X., Jansson P. A., Pellme F., Laakso M. and Smith U. 2005 Effect of the interleukin-6 (-174) g/c promoter polymorphism on adiponectin zand insulin sensitivity. *Obes. Res.* **13**, 813–817.

Zhang D., Zheng H., Zhou Y., Tang X., Yu B. and Li J. 2007 Association of IL-1beta gene polymorphism with cachexia from locally advanced gastric cancer. *BMC Cancer* **7**, 45.

Zhang D., Zhou Y., Wu L., Wang S., Zheng H., Yu B. *et al.* 2008 Association of IL-6 gene polymorphisms with cachexia susceptibility and survival time of patients with pancreatic cancer. *Ann. Clin. Lab. Sci.* **38**, 113–119.

Received 19 April 2010, in final revised form 16 August 2010; accepted 18 August 2010
Published on the Web: 19 May 2011