

Body mass index change in gastrointestinal cancer and chronic obstructive pulmonary disease is associated with Dedicator of Cytokines 1

Merry-Lynn Noelle McDonald^{1*}, Sungho Won², Manuel Mattheisen³, Peter J. Castaldi^{1,4}, Michael H. Cho^{1,5}, Erica Rutten⁶, Megan Hardin^{1,5}, Wai-Ki Yip⁷, Stephen I. Rennard⁸, David A. Lomas⁹, Emiel F.M. Wouters^{6,10}, Alvar Agusti¹¹, Richard Casaburi¹², Christoph P. Lange⁷, George O'Connor^{13,14}, Craig P. Hersh^{1,5} & Edwin K. Silverman^{1,5}

¹Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; ²Department of Public Health Science, Seoul National University, Seoul, South Korea; ³Department of Biomedicine and Centre for integrative Sequencing (iSEQ), Aarhus University, Aarhus, Denmark; ⁴Division of Primary Care and Internal Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; ⁵Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; ⁶CIRO+, Centre of Expertise for Chronic Organ Failure, Horn, The Netherlands; ⁷Department of Biostatistics, Harvard School of Public Health, Boston, MA, USA; ⁸Department of Medicine, Nebraska Medical Center, Omaha, Nebraska, USA; ⁹UCL Respiratory, Division of Medicine, University College London, London, UK; ¹⁰Department of Respiratory Medicine, Maastricht University Medical Center Maastricht, Maastricht, The Netherlands; ¹¹Thorax Institute, Hospital Clinic-IDIBAPS, CIBERES, University of Barcelona, Barcelona, Spain; ¹²Rehabilitation Clinical Trials Center, Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA, USA; ¹³The National Heart, Lung, and Blood Institute's Framingham Heart Study, Framingham, MA, USA; ¹⁴Section of Pulmonary, Allergy, and Critical Care Medicine, Department of Medicine, Boston University School of Medicine, Boston, MA, USA

Abstract

Background There have been a number of candidate gene association studies of cancer cachexia-related traits, but no genome-wide association study (GWAS) has been published to date. Cachexia presents in patients with a number of complex traits, including both cancer and COPD. The objective of the current investigation was to search for a shared genetic aetiology for change in body mass index (Δ BMI) among cancer and COPD by using GWAS data in the Framingham Heart Study.

Methods A linear mixed effects model accounting for age, sex, and change in smoking status was used to calculate Δ BMI in participants over 40 years of age with three consecutive BMI time points ($n=4162$). Four GWAS of Δ BMI using generalized estimating equations were performed among 1085 participants with a cancer diagnosis, 204 with gastrointestinal (GI) cancer, 112 with lung cancer, and 237 with COPD to test for association with 418 365 single-nucleotide polymorphisms (SNPs).

Results Two SNPs reached a level of genome-wide significance ($P < 5 \times 10^{-8}$) with Δ BMI: (i) rs41526344 within the *CNTN4* gene, among COPD cases ($\beta=0.13$, $P=4.3 \times 10^{-8}$); and (ii) rs4751240 in the gene Dedicator of Cytokines 1 (*DOCK1*) among GI cancer cases ($\beta=0.10$, $P=1.9 \times 10^{-8}$). The *DOCK1* SNP association replicated in the Δ BMI GWAS among COPD cases ($\beta_{\text{meta-analysis}}=0.10$, $P_{\text{meta-analysis}}=9.3 \times 10^{-10}$). The *DOCK1* gene codes for the dedicator of cytokines 1 protein, which has a role in myoblast fusion.

Conclusions In sum, one statistically significant common variant in the *DOCK1* gene was associated with Δ BMI in GI cancer and COPD cases providing support for at least partially shared aetiology of Δ BMI in complex diseases.

Keywords GWAS; COPD; Cancer; Longitudinal; BMI; Cachexia

Received: 28 June 2016; Revised: 26 September 2016; Accepted: 2 November 2016

*Correspondence to: Merry-Lynn Noelle McDonald, Division of Pulmonary Allergy and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, AL, USA. Email: mmcdonald@uab.edu

Introduction

Despite the success of genome-wide association studies (GWASes) at identifying thousands of associations with

hundreds of complex diseases and traits,¹ there has been no published GWAS investigating a cancer cachexia-related trait. Although there have been a number of published candidate-gene association studies investigating cancer

cachexia-related traits (reviewed in Refs^{2,3}), candidate-gene association studies are limited to known biology and do not take advantage of the agnostic, hypothesis-free approach of genome scans. Thus, the cancer cachexia community would benefit from GWASes in order to discover new genes and pathways involved in the aetiology of cachexia.

Body mass index (BMI) is a highly heritable cross-sectional measure that is frequently employed to monitor changes in body composition in epidemiological studies. Over 100 genetic loci have been associated with BMI.^{4,5} Changes in BMI (Δ BMI) have also been shown to be heritable with estimates ranging from 0.14 to 0.86.^{6,7} At one end of the BMI spectrum, obesity is a known risk factor for a number of complex diseases. At the other end of the spectrum, patients who lose weight are also at increased risk of mortality, which is the impetus for investigating muscle wasting and cachexia.⁸ Cachexia can be characterized in part as a rapid change in weight, including loss of fat-free muscle, as part of the pathology of an illness.⁸ Cachexia is often thought of with respect to cancer; however, it occurs in patients suffering from many chronic, complex diseases, including chronic obstructive pulmonary disease (COPD), congestive heart failure, AIDS, and cystic fibrosis, among others.^{8,9} In fact, the number of COPD patients with cachexia was recently estimated to be 1.4 times higher than the number of cancer patients with cachexia.¹⁰ Regardless of the primary complex disease diagnosis, cachexia is associated with poor prognosis and an increased burden on the healthcare system.^{8,11}

In the current report, we begin to fill the gap in reported GWASes investigating cachexia-related traits. We analysed genetic data available via public access (dbGaP) from the Framingham Heart Study (FHS).¹² In the current manuscript, we integrated data from GWAS of Δ BMI in cancer and COPD cases to search for overlapping genetic variants.

Materials and methods

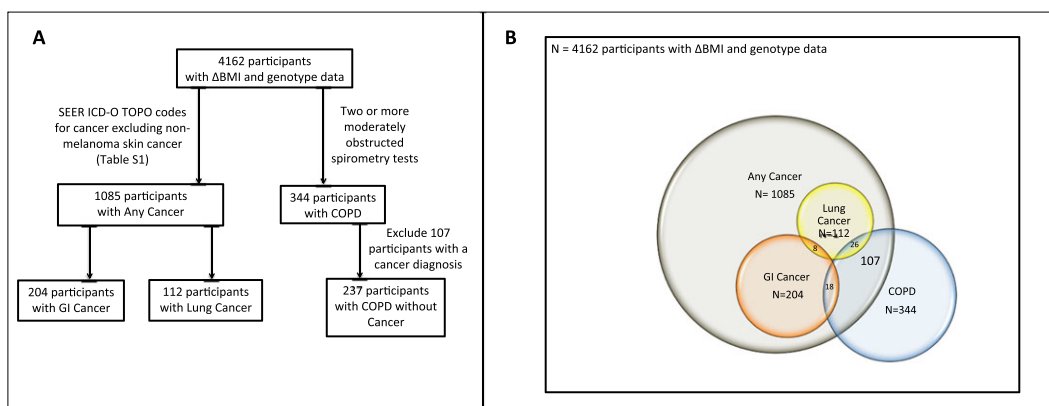
Study population

Ethics approval was obtained for the proposed analyses from the Brigham and Women's Hospital Institutional Review Board and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Phenotype and genotype data from FHS were used in the analyses. The FHS is a longitudinal population-based study of individuals from Framingham, MA.¹² FHS genotype and phenotype data were downloaded through authorized accession obtained from National Institutes of Health Data Access Committee of dbGaP phs000007.v16.p6. Genotype data from the Affymetrix 500 k mapping array plus the Affymetrix 50 k supplemental array (phs000342.v4.p6) were used in the analyses.

Generating change in body mass index phenotype in cancer and chronic obstructive pulmonary disease cases

Figure 1A depicts a flowchart illustrating the number of subjects included in the analyses with genotype, Δ BMI, and additional phenotype (cancer and/or COPD diagnoses). Using the FHS data, Δ BMI was calculated by using the last three consecutive time points (~2–4 years apart) for individuals over 40 years of age who participated in the Original and Offspring Cohorts by using a random slope and intercept linear mixed effects model accounting for age, sex, and change in smoking status as fixed effects. COPD diagnosis was generated by using spirometry¹³ reported at visits 16, 17, and 19 in the FHS Original Cohort and

Figure 1 (A) Flowchart of number of subjects included in analyses with genotype, change in body mass index and additional phenotype data. (B) Venn diagram representing overlap between all subjects with change in body mass index, cancer, and chronic obstructive pulmonary disease diagnoses. Counts are based on the number of participants with genotype data and change in body mass index variable.



visits 5, 6, 7, and 8 in the FHS Offspring Cohort. An individual was coded as having COPD if they had at least two moderately obstructed (or worse) spirometry tests ($FEV_1 < 80\%$ and $FEV_1/FVC < 0.7$) that did not improve at a later visit ($n=344$). An individual was coded as having any cancer if they reported a cancer diagnosis as coded with SEER ICD-O topography (TOPO) codes at any time during the study ($n=1085$). Please see Table S1 for specific details on the TOPO codes used. Overall, 4162 participants had information to calculate Δ BMI and genotype data. Of these, 1085 had a cancer diagnosis, 344 had COPD based on lung function, 204 had a GI cancer diagnosis, and 112 had a lung cancer diagnosis. A total of 237 COPD cases did not also have a concurrent cancer diagnosis based on TOPO codes.

Genome-wide association studies of change in body mass index

Genotyping quality control procedures were used to ensure high-quality data for association testing. The Affymetrix 500k and 50k data were cleaned separately and then combined prior to association testing. Briefly, DNA samples were excluded if missing more than 3% of genotypes, if reported familial relationships did not agree with those estimated from the genotypes, exhibited excess heterozygosity or homozygosity, or were outliers by using principal components analysis to detect population substructure. Single-nucleotide polymorphisms (SNPs) that failed genotyping in more than 5% of the samples or with Hardy–Weinberg equilibrium P -values less than 1×10^{-7} were removed. SNP analyses were restricted to autosomal variants with minor allele frequencies greater than 5%. A total of 4 GWAS (within COPD, all cancer, lung cancer, and GI cancer subjects) were performed by using generalized estimating equations accounting for family structure in FHS to test for association between 418 365 SNPs and Δ BMI by using the R library GWAF.¹⁴ All GWAS findings were further adjusted by using genomic control to control for population substructure.¹⁵ Meta-analyses were

performed between independent samples from FHS cancer cases and FHS COPD cases without cancer, assuming a fixed effects model by using meta software.¹⁶ Meta-analysis results with $P_{\text{heterozygosity}} < 0.1$ were excluded. Regional association plots were generated by using LocusZoom.¹⁷ HaploReg v4.1 was used to assess the relationship between significant GWAS SNPs and epigenomic annotations in cell types from the Roadmap Epigenomics and ENCODE projects.¹⁸ The results with $P < 5 \times 10^{-8}$ were considered statistically/genome-wide significant (GWS) accounting both for the number of variants tested and the linkage disequilibrium (LD) structure of the genome.^{19,20}

Results

Cancer and chronic obstructive pulmonary disease population characteristics

Only subjects over the age of 40 with at least 3 consecutive BMI measurements in FHS were considered for the analyses. There was some overlap in cancer and COPD diagnoses in the population (Figure 1B). Among FHS participants who had a COPD diagnosis, 107 (31.1%) also reported a cancer diagnosis of which 18 (5.2%) had GI cancer and 26 (7.6%) had lung cancer. In the current genetic analysis, COPD cases with a cancer diagnosis were excluded from the COPD Δ BMI GWAS to create an independent case population for meta-analyses.

Table 1 summarizes the characteristics of cancer and COPD cases from the FHS included in the analyses. A total of 4162 FHS participants over the age of 40 at baseline BMI measurement had three consecutive time points available to generate Δ BMI. Among these FHS participants, 237 had COPD based on spirometry without concurrent reported cancer diagnoses, 1085 had some type of cancer (except non-melanoma skin cancer), 204 had GI cancer, and 112 had lung cancer (Figure 1). The percentage of men was comparable between the COPD cases and the

Table 1 Characteristics of cancer and chronic obstructive pulmonary disease cases in Framingham Heart Study used in analyses

	Framingham Heart Study (FHS)				
	All	Any cancer	COPD ^a	GI cancer	Lung cancer
<i>n</i>	4162	1085	237	204	112
Sex (% male)	45.2	51.8	45.6	52	57.1
Median initial BMI (IQR)	26.8 (6.0)	27 (5.7)	26.4 (6.1)	26.7 (5.6)	25.8 (4.9)
Median initial age (IQR)	62 (19)	66 (17)	61 (15)	69 (19)	66 (15.2)
Median follow-up time (IQR)	9 (4)	8 (4)	8 (6)	7 (5)	7 (4)
Median Δ BMI (IQR)	0.01 (0.2)	0.01(0.1)	0.03 (0.2)	-0.02 (0.1)	0.008 (0.1)

Δ BMI, change in body mass index; COPD, chronic obstructive pulmonary disease; GI, gastrointestinal; IQR, interquartile range.

^aChronic obstructive pulmonary disease cases without any cancer. Chronic obstructive pulmonary disease in FHS was coded by using lung function testing. Initial age and BMI in FHS are based the first of three consecutive time points an individual participated in FHS.

total subset with Δ BMI, whereas there were more men with a cancer diagnosis in the FHS data than total subset with Δ BMI (Table 1). The lung cancer population had the highest percentage of men (57.1%). Baseline BMI was comparable between the total FHS with Δ BMI, COPD, and cancer subsets. The FHS participants with a cancer diagnosis were older (median: 66 years) than those with COPD without cancer (median: 61 years) and the collective FHS population with Δ BMI (median: 62 years). The GI cancer case subset had the oldest median baseline age of 69 years (Table 1). The follow-up time was comparable among the COPD, cancer, and total populations with Δ BMI (follow-up time range: 7–9 years). Overall, most participants' BMI increased slightly (median Δ BMI: 0.01) during the interval used to evaluate BMI. On average, the COPD population's BMI increased the most (median Δ BMI: 0.03), and the GI cancer population was the only population that lost BMI overall (median Δ BMI: -0.02).

Genome-wide association studies of change in body mass index in cancer and chronic obstructive pulmonary disease cases

The quantile–quantile and Manhattan plots summarizing the results from the individual Δ BMI GWASes among cancer and COPD cases are presented in Figures S1–S4. All SNP results with suggestive associations ($P < 5 \times 10^{-5}$) are presented in Tables S2–S5. No SNP reached a level of GWS ($P < 5 \times 10^{-8}$) in the GWAS of Δ BMI among all cancer cases (Figure S1). However, two SNPs achieved GWS with Δ BMI separately among COPD or GI cancer cases. More specifically, rs41526344, within the *CNTN4* gene, was significantly associated with Δ BMI among COPD cases ($\beta = 0.13$, $P = 4.3 \times 10^{-8}$). *CNTN4* codes for contactin 4, a neuronal network axon-associated cell adhesion molecule implicated in the genetics of neuropsychiatric disorders.²¹ Further, rs4751240 was significantly associated with Δ BMI among GI cancer cases ($\beta = 0.10$, $P = 1.9 \times 10^{-8}$) and is located within the Dedicator of Cytokinesis 1 (*DOCK1*) gene, which codes for the dedicator of cytokinesis 1 protein and has a role in myoblast fusion.²² The *DOCK1* SNP, rs4751240, has a minor allele frequency of 7% in the GI cancer population, and under Hardy–Weinberg equilibrium, we would expect that 0.5% of the population would be homozygous for the variant and 13% would be heterozygous. For the rs41526344 SNP within the *CNTN4* gene, we would expect 0.8% of the population to be homozygous and 14.6% to be heterozygous based on the minor allele frequency in the COPD population. The observed genotype frequencies did not differ significantly from those predicted based on the Hardy–Weinberg equilibrium test for these variants or any variant reported in this study.

Top single-nucleotide polymorphisms associated with change in body mass index among both cancer and chronic obstructive pulmonary disease cases

To test the hypothesis that shared variants are associated with Δ BMI among cancer and COPD cases, meta-analyses of the Δ BMI GWASes between cancer and COPD cases were performed. For example, separate meta-analyses were run between the Δ BMI GWASes from GI cancer and COPD cases, between the Δ BMI GWASes from lung cancer cases and COPD cases, and so forth. All meta-analysis results with suggestive associations ($P < 5 \times 10^{-5}$) are presented in Tables S8–S10. No variant achieved a level of GWS in the meta-analysis between all cancer cases and COPD cases. The top SNP rs188981 ($\beta_{\text{meta-analysis}}: -0.025$, $P_{\text{meta-analysis}} = 6.6 \times 10^{-6}$) is located in an intergenic region on chromosome 3 between the genes *SUCNR1* and *MBLN1* (Figure 2A and Table S6). The GWS association observed among the *DOCK1* SNP, rs4751240, and Δ BMI in GI cancer was replicated in the COPD Δ BMI GWAS ($\beta_{\text{COPD}}: 0.08$, $P_{\text{COPD}} = 0.014$, $\beta_{\text{meta-analysis}}: 0.1$, $P_{\text{meta-analysis}} = 9.3 \times 10^{-10}$; Table S7). This β accounts for a mean increase of 0.1 kg/m² BMI per year in GI cancer cases and of 0.08 kg/m² BMI per year in COPD cases after taking age, sex, and change in smoking status into account for each copy of the rs4751240 minor allele (Figure 3). The regional association plot (Figure 2B) demonstrates that the genotyped rs4751240 SNP is intronic within the coding region of *DOCK1* and is not in LD with other genotyped variants. When HaploReg was used to examine LD of variants with rs4751240 by using the 1000 Genomes Project haplotypes, only one other variant in the region is in high LD with this SNP (Table S8). The investigation using HaploReg also revealed that rs4751240 alters a motif²³ corresponding to the tumour suppressor Nkx2-8.^{22,23} We also identified an association in the meta-analysis of Δ BMI between lung cancer and COPD cases near the *FAM73B* gene (rs7042889, $\beta_{\text{meta-analysis}}: -0.14$, $P_{\text{meta-analysis}} = 1.4 \times 10^{-7}$; Table S10). The HaploReg investigation revealed that rs7042889 is in high LD with eight SNPs that alter regulatory motifs, including rs7873667 which alters a number of motifs including several forkhead box (Fox) motifs (Table S10).

Discussion

In summary, we report findings from the first GWAS of a cachexia-related trait, Δ BMI, among cancer and COPD cases. We found one SNP, rs41526344, significantly associated with Δ BMI among COPD cases in the *CNTN4* gene but were unable to replicate it. The *DOCK1* SNP rs4751240 was significantly associated with Δ BMI among GI cancer cases and replicated among COPD cases. We also identified a nearly significant

Figure 2 Regional association plots of the top regions identified in the meta-analysis between cancer and chronic obstructive pulmonary disease cases: (A) The region on chromosome 3 where the top variant from the meta-analysis investigating change in body mass index among any cancer and chronic obstructive pulmonary disease cases rs188981 ($\beta = -0.025$, $P = 6.0 \times 10^{-6}$) is located in an intergenic region between the genes *SUCNR1* and *MBLN1*. (B) Displays the *DOCK1* region on chromosome 9, where the top variant rs4751240 ($\beta = 0.1$, $P = 9.3 \times 10^{-10}$) in the meta-analysis between investigating change in body mass index among gastrointestinal cancer and chronic obstructive pulmonary disease cases is located.

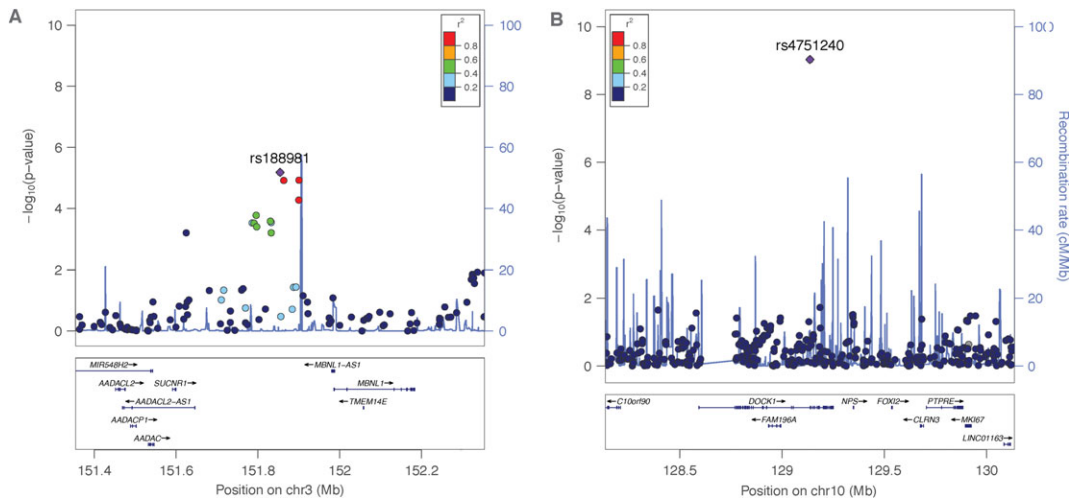
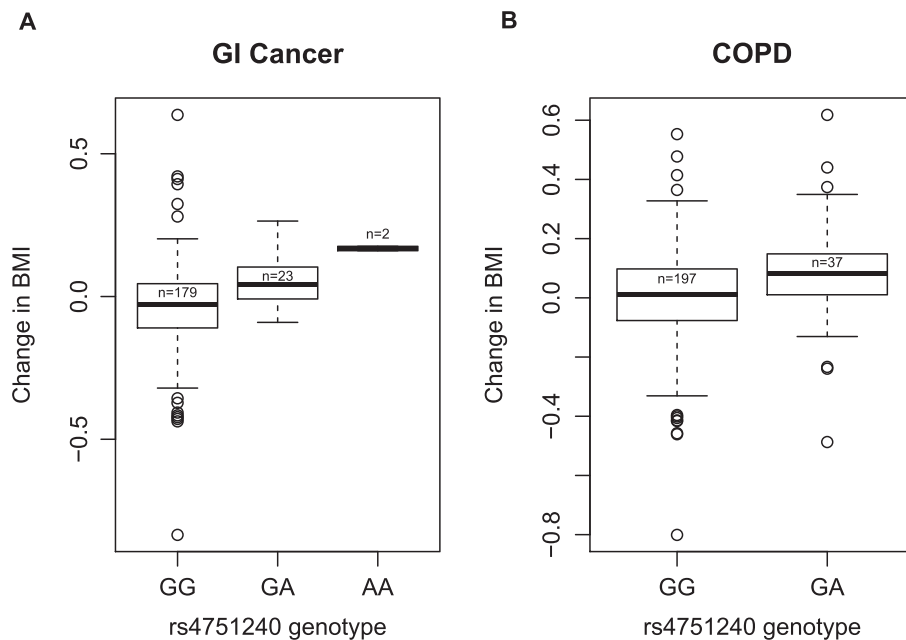


Figure 3 Boxplot of change in body mass index stratified by *DOCK1* SNP rs4751240 genotype between (A) gastrointestinal cancer cases and (B) and chronic obstructive pulmonary disease cases.



association with rs7042889 in the meta-analysis of Δ BMI between lung cancer and COPD cases near the *FAM73B* gene.

The rs4751240 SNP in the *DOCK1* gene achieved a level of GWS ($P < 5 \times 10^{-8}$) in the analysis of Δ BMI among GI cancer cases and replicated nominally in the analyses among COPD cases. *DOCK1* is a guanine nucleotide exchange factor,

catalysing the exchange of GDP to GTP to activate Rho proteins.²⁴ In mouse models, knockout of *DOCK1* is embryonic lethal with a dramatic reduction of skeletal muscle tissues attributed to a deficiency in myoblast fusion.²² Myoblast fusion is also critical for satellite cell-mediated muscle regeneration in adults.²⁵ As cachexia can be the result of both

increased muscle breakdown and decreased muscle production, genetic variation in *DOCK1* may influence muscle regeneration. The *DOCK1* SNP, rs4751240, is an intronic variant. SNPs in non-coding regions of the genome may directly alter or may be in LD with genetic variants that alter regulatory regions such as transcription factor or enhancer binding sites and in turn have an effect on gene expression and protein abundance.²⁶ The rs4751240 SNP is within a motif for the tumour suppressor Nkx2-8, whose mechanism of tumour suppression is by up-regulating FOXO3a protein encoded by the *FOXO3* gene.²⁷ The Fox class O (FoxO) transcription factor family members FOXO1 and FOXO3 are well known to play a role in energy metabolism, protein breakdown, regulation of muscle mass, and adaptation to exercise.²⁸ All FoxOs are activated in response to insulin stimulation and/or oxidative stress by phosphorylation at three evolutionarily conserved sites.²⁸ Interestingly, the top SNP associated with Δ BMI in the meta-analysis of lung cancer and COPD cases is in high LD with rs7873667 (Table S10) that disrupts several Fox transcription factors, including FoxO. In a recent mega-GWAS, rs9400239 in *FOXO3* was significantly associated with cross-sectional BMI ($\beta=0.0019$, $P=1.61 \times 10^{-8}$) in individuals of European ancestry, further supporting the role of this pathway in determining BMI.⁴

The top finding in the meta-analysis of Δ BMI between all cancer and COPD cases is intergenic to *SUCNR1* and *MBLN1*. *SUCNR1* codes for the succinate receptor 1 gene, which is a G-protein coupled receptor for succinate, an intermediate of the citric acid cycle.²⁹ *MBLN1* codes for the muscleblind-like splicing regulator 1, which plays a role in myotonic dystrophy and knockout of the gene in the mouse leads to muscle abnormalities and cataracts.³⁰ This result, however, fails to achieve a level of genome-wide significance. The power of the analysis may be limited both by the sample size and heterogeneity of Δ BMI trajectories among different types of cancer. The analyses that were performed among different cancer types further reduced the sample size but may have increased the homogeneity of the Δ BMI trajectory, thereby increasing the power to detect the significant association in the GI cancer group.

We identified one variant, rs41526344, significantly associated with Δ BMI among COPD cases in the *CNTN4* gene, coding for contactin-4. Contactins are a family of neural immunoglobulin cell adhesion molecules (IgCAMs) with six N-terminal immunoglobulin repeats followed by four fibronectin type III repeats. The prototypical contactin is contactin-1 with shared amino acid identity ranging from 33 to 52% identity with contactin-4 depending on the domain.²¹ Genetic variations in *CNTN4*, *CNTN5*, and *CNTN6* have been associated with autism spectrum disorders.³¹ Increased signal transduction via the mTOR signal transduction pathway is one mechanism common to many autism spectrum disorder genes including contactins.³¹ Interestingly, decreased mTOR signalling is observed during fasting and disease as muscle growth and atrophy depends on the mTOR pathway.³²

Further, mutations in *CNTN1* cause a familial form of the lethal congenital myopathy, a disease whose hallmark is low muscle tone.³³ In the current study, we identified a significant association with a *CNTN4* SNP with Δ BMI among COPD cases; however, we were not able to replicate the findings among cancer cases.

There have been a number of candidate gene association studies investigating the genetics of cancer cachexia-related traits such as low BMI, low FFMI, weight loss, and muscle strength, but no GWAS has been reported to our knowledge. Systematic reviews of the genetics of cancer cachexia highlighted genes involved in inflammatory response regulation, homeostasis, pathways directing muscle and fat metabolism, and appetite regulation.^{2,3} Among COPD cases, muscle quadriceps muscle strength and low FFMI candidate gene studies have reported associations with the Angiotensin Converting Enzyme gene,³⁴ Bradykinin Receptor gene,³⁵ IL1 β ,³⁶ IL6,³⁶ TNF α ,³⁶ and vitamin D receptor.³⁷ Wan and colleagues from our research group³⁸ reported GWAS findings investigating two continuous outcomes, BMI and FFMI, in COPD cases. They found several SNPs tagging the fat-mass and obesity (*FTO*) gene significantly associated (meta-analysis: $P < 5 \times 10^{-7}$) with reduced BMI and FFMI in COPD cases. Single-nucleotide polymorphisms in the *FTO* gene have been repeatedly associated with BMI³⁹⁻⁴⁴ and diabetes.⁴⁵ It is interesting to note that we did not find statistically significant associations with any known cachexia candidate genes. This may indicate that the genetic aetiology of longitudinal BMI is different than cross-sectional BMI; however, larger sample sizes will be required to clarify this issue. The current report begins to fill the void of reported GWASes investigating cachexia-related traits and provides a foundation for expanding this work to larger case populations with more extensive phenotyping for monitoring cachexia.

In the current report, we took advantage of a publicly available dataset to test the hypothesis that a shared genetic aetiology exists for Δ BMI among cancer and COPD cases. We considered SNPs with $P < 5 \times 10^{-8}$, the generally accepted threshold for statistical significance in GWAS, as statistically significant because it has been shown to account both for the number of variants tested and the LD structure of the genome.^{19,20} Further, the Bonferroni adjusted threshold for 418 365 SNPs tested in four GWASes is $\alpha=3.0 \times 10^{-8}$, and the finding with *DOCK1* among GI cancer cases is also below this more conservative level of statistical significance ($P=1.9 \times 10^{-8}$). We do acknowledge that the sample sizes became relatively small when we examined individual groups of cancer patients. Despite this limitation, the rs4751240 finding in *DOCK1* did reach a level of genome-wide significance among GI cancer cases, and we were able to replicate the finding among COPD cases contributing to a meta-analysis of $P=9.3 \times 10^{-10}$. This finding supports the hypothesis that a shared genetic aetiology exists for Δ BMI among GI cancer and COPD cases and also underscores the need for further

investigations into larger cancer and COPD populations to increase the power to detect additional signals. Further, our investigation was underpowered for additional genetic analyses such as epistasis. In future investigations, we plan to investigate the role of epistasis in cachexia by, for example, testing for significant genetic interactions between the *DOCK1* SNP identified in the current analyses and variants in other candidate genes whose role in cachexia has been implicated.

Further limitations of the analyses include an inability to address the temporal relationship between disease diagnoses and Δ BMI in our analyses. It is possible, for example, that an individual received a cancer diagnosis many years before the initial BMI time point used to generate the Δ BMI phenotype in FHS. We performed our analyses in a longitudinal, population-based cohort and screened for participants over the age of 40 and only considered the last three consecutive BMI measurements reported among cancer and COPD cases. The median age for the first BMI measurement included in the GI cancer analysis was 69 with a median follow-up period of 7 years (Table 1). With the focus on the last three consecutive time points with BMI data available in FHS combined with the advanced age of the population under investigation, the majority of cancer diagnoses would most likely have been made either prior or during the window we investigated Δ BMI. Although timing of diagnosis of cancer relative to Δ BMI is important, even more important issues are the stage, extent, and treatment of the cancer, including consideration of course of chemotherapy and any resultant toxicity, relative to Δ BMI. We plan to pursue these more detailed characterizations in future studies. Cancers are likely present for years before diagnoses are made, and the pre-diagnosis duration is typically unknown. We think that the fact that we have found interesting genetic association results with these limitations argues that there are likely important signals to find.

The current report has many limitations. We mined a publically available study, the FHS, to investigate Δ BMI among COPD and cancer cases. The FHS participants had BMI measured every 2–4 years. The ideal study design would ascertain patients for longitudinal assessments of cachexia, including measuring BMI and/or more precise measurements of muscle mass every 3–6 months in addition to other characteristics established in the cachexia consensus definition.⁸ Further, our sample sizes became small when we restricted analyses to cancer and COPD subsets of the population. As we used a hypothesis-free approach to search for genetic variants associated with Δ BMI among cancer and COPD cases, replicating the finding in independent populations ascertained for cachexia will be a crucial next step.

The current analysis demonstrates evidence for the involvement of the *DOCK1* gene with Δ BMI among both GI cancer and COPD cases. The current investigation has highlighted genes known for their involvement in energy metabolism and muscle maintenance as associated with Δ BMI. Future directions will include collection and analysis of gene expression

data to identify expression biomarkers associated with Δ BMI among COPD and cancer cases in addition to more information on cancer diagnosis, stage, extent, and treatment. Our future efforts will be directed at analysing additional longitudinal data characterizing both genetic and gene expression to gain further insight on biomarkers that can be used to monitor the development and prognosis of cachexia.

Acknowledgements

The authors certify that they comply with the ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015.⁴⁶ A portion of the results of this manuscript have been published in the form of an abstract: the American Society of Human Genetics Conference, Baltimore, MD, 2015. The FHS is conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with Boston University (contract no. N01-HC-25195). This manuscript does not necessarily reflect the opinions or views of the FHS, Boston University, or NHLBI. SHARe Illumina genotyping was provided under an agreement between Illumina and Boston University.

This study was funded by the National Institutes of Health—National Heart, Lung, and Blood Institute (N02-HL-64278, N01 HC 25195, P01 HL105339, R01 HL111759, R01 HL113264, K99 HL121087); Parker B Francis Foundation.

Online supplementary material

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Quantile-Quantile and Manhattan plots of GWAS results of the FHS Δ BMI phenotype in any cancer cases

Figure S2. Quantile-Quantile and Manhattan plots of GWAS results of the FHS Δ BMI phenotype in COPD cases

Figure S3. Quantile-Quantile and Manhattan plots of GWAS results of the FHS Δ BMI phenotype in GI cancer cases

Figure S4. Quantile-Quantile and Manhattan plots of GWAS results of the FHS Δ BMI phenotype in lung cancer cases

Table S1. ICD-O TOPO codes used to classify cancer cases.

Table S2. Suggestive results ($P < 5 \times 10^{-5}$) from the GWAS of Δ BMI in any cancer cases

Table S3. Suggestive results ($P < 5 \times 10^{-5}$) from the GWAS of Δ BMI in GI cancer cases

Table S4. Suggestive results ($P < 5 \times 10^{-5}$) from the GWAS of Δ BMI in COPD cases

Table S5. Suggestive results ($P < 5 \times 10^{-5}$) from the GWAS of Δ BMI in lung cancer cases

Table S6. Suggestive results ($P < 5 \times 10^{-5}$) from the GWAS of Δ BMI in breast cancer cases

Table S7. Suggestive results ($P < 5 \times 10^{-5}$) from the GWAS of Δ BMI in prostate cancer cases

Table S8. Suggestive results ($P < 5 \times 10^{-5}$) from the meta-analysis GWAS of Δ BMI in any cancer cases with GWAS of Δ BMI COPD cases.

Table S9. Suggestive results ($P < 5 \times 10^{-5}$) from the meta-analysis GWAS of Δ BMI in GI cancer cases with GWAS of Δ BMI COPD cases.

Table S10. Suggestive results ($P < 5 \times 10^{-5}$) from the meta-analysis GWAS of Δ BMI in lung cancer cases with GWAS of Δ BMI COPD cases.

Conflict of Interest

M.-L.N. McDonald declares that she has no conflict of interest. S. Won declares that he has no conflict of interest. M. Mattheisen declares that he has no conflict of interest. P.J. Castaldi declares that he has no conflict of interest. M. H. Cho declares that he has no conflict of interest. E. Rutten declares that she has no conflict of interest. M. Hardin declares that she has no conflict of interest. W.-K. Yip declares that he has no conflict of interest. S. Rennard is the Richard and Margaret Larson Professor of Pulmonary Research at University of Nebraska Medical Center and has a number of relationships with companies who provide products and/or services relevant to outpatient management of chronic obstructive pul-

monary disease, including A2B Bio, Almirall, APT, AstraZeneca, Boehringer Ingelheim, Chiesi, CME Incite, CSL Behring, Dailchi Sankyo, Decision Resources, Dunn Group, Easton Associates, Forest, Gerson, GlaxoSmithKline, Johnson and Johnson, Medimmune, Novartis, Novis, Nycomed, Otsuka, Pearl, Pfizer, PriMed, Pulmatrix, Roche, Takeda, and Theravance; these relationships include serving as a consultant, advising regarding clinical trials, speaking at continuing medical education programmes, and performing funded research both at basic and clinical levels. S. Rennard is employed by AstraZeneca in which he owns shares. S. Rennard does not own any stock in any other pharmaceutical companies. D.A. Lomas has received grant support, honoraria, and consultancy fees from GlaxoSmithKline who sponsored the ECLIPSE study. E.F.M. Wouters declares that he has no conflict of interest. A. Agusti reports grants and personal fees from Astra-Zeneca, GSK, MSD, and Menarini and personal fees from Novartis, TEVA, and Chiesi outside the submitted work. R. Casaburi has received honoraria and consulting fees from Boehringer-Ingelheim, GlaxoSmithKline, Novartis, Astellas, and Astra Zeneca. C. Lange declares that he has no conflict of interest. G. O'Connor declares that he has no conflict of interest. C.P. Hersh declares that he has no conflict of interest. E.K. Silverman has received honoraria and consulting fees from Merck, grant support and consulting fees from GlaxoSmithKline, and honoraria and travel support from Novartis in the past 3 years.

References

- Manolio TA. Bringing genome-wide association findings into clinical use. *Nat Rev Genet* 2013;**14**:549–558.
- Tan BH, Ross JA, Kaasa S, Skorpen F, Fearon KC. Identification of possible genetic polymorphisms involved in cancer cachexia: a systematic review. *J Genet* 2011;**90**:165–177.
- Johns N, Tan BH, MacMillan M, Solheim TS, Ross JA, Baracos VE, et al. Genetic basis of interindividual susceptibility to cancer cachexia: selection of potential candidate gene polymorphisms for association studies. *J Genet* 2014;**93**: 893–916.
- Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature* 2015;**518**: 197–206.
- Winkler TW, Justice AE, Graff M, Barata L, Feitosa MF, Chu S, et al. The influence of age and sex on genetic associations with adult body size and shape: a large-scale genome-wide interaction study. *PLoS Genet* 2015;**11**:e1005378.
- Austin MA, Friedlander Y, Newman B, Edwards K, Mayer-Davis EJ, King MC. Genetic influences on changes in body mass index: a longitudinal analysis of women twins. *Obes Res* 1997;**5**:326–331.
- Hunt MS, Katzmarzyk PT, Perusse L, Rice T, Rao DC, Bouchard C. Familial resemblance of 7-year changes in body mass and adiposity. *Obes Res* 2002;**10**:507–517.
- Evans WJ, Morley JE, Argiles J, Bales C, Baracos V, Guttridge D, et al. Cachexia: a new definition. *Clin Nutr* 2008;**27**:793–799.
- Morley JE, Thomas DR, Wilson M-MG. Cachexia: pathophysiology and clinical relevance. *Am J Clin Nutr* 2006;**83**: 735–743.
- von Haehling S, Anker SD. Prevalence, incidence and clinical impact of cachexia: facts and numbers—update 2014. *J Cachexia Sarcopenia Muscle* 2014;**5**: 261–263.
- Arthur ST, Noone JM, Van Doren BA, Roy D, Blanchette CM. One-year prevalence, comorbidities and cost of cachexia-related inpatient admissions in the USA. *Drugs Context* 2014;**3**:212265.
- Govindaraju DR, Cupples LA, Kannel WB, O'Donnell CJ, Atwood LD, D'Agostino RB Sr, et al. Genetics of the Framingham Heart Study population. *Adv Genet* 2008;**62**:33–65.
- Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999;**159**:179–187.
- Chen MH, Yang Q. GWAF: an R package for genome-wide association analyses with family data. *Bioinformatics* 2010;**26**: 580–581.
- Devlin B, Roeder K. Genomic control for association studies. *Biometrics* 1999;**55**: 997–1004.
- Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010;**26**:2190–2191.
- Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Glied TP, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 2010;**26**:2336–2337.

18. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;**40**:D930–934.
19. Dudbridge F, Gusnanto A. Estimation of significance thresholds for genomewide association scans. *Genet Epidemiol* 2008;**32**:227–234.
20. A haplotype map of the human genome. *Nature* 2005;**437**:1299–1320.
21. Zuko A, Bouyain S, van der Zwaag B, Burbach JP. Contactins: structural aspects in relation to developmental functions in brain disease. *Adv Protein Chem Struct Biol* 2011;**84**:143–180.
22. Laurin M, Fradet N, Blangy A, Hall A, Vuori K, Cote JF. The atypical Rac activator Dock180 (Dock1) regulates myoblast fusion in vivo. *Proc Natl Acad Sci U S A* 2008;**105**:15446–15451.
23. Kheradpour P, Kellis M. Systematic discovery and characterization of regulatory motifs in ENCODE TF binding experiments. *Nucleic Acids Res* 2014;**42**:2976–2987.
24. Gadea G, Blangy A. Dock-family exchange factors in cell migration and disease. *Eur J Cell Biol* 2014;**93**:466–477.
25. Jansen KM, Pavlath GK. Molecular control of mammalian myoblast fusion. *Methods Mol Biol* 2008;**475**:115–133.
26. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 2012;**337**:1190–1195.
27. Yu C, Zhang Z, Liao W, Zhao X, Liu L, Wu Y, et al. The tumor-suppressor gene Nkx2.8 suppresses bladder cancer proliferation through upregulation of FOXO3a and inhibition of the MEK/ERK signaling pathway. *Carcinogenesis* 2012;**33**:678–686.
28. Eijkelenboom A, Burgering BM. FOXOs: signalling integrators for homeostasis maintenance. *Nat Rev Mol Cell Biol* 2013;**14**:83–97.
29. Gilissen J, Jouret F, Piroette B, Hanson J. Insight into SUCNR1 (GPR91) structure and function. *Pharmacol Ther* 2016;**159**:56–65.
30. Suenaga K, Lee KY, Nakamori M, Tatsumi Y, Takahashi MP, Fujimura H, et al. Muscleblind-like 1 knockout mice reveal novel splicing defects in the myotonic dystrophy brain. *PLoS One* 2012;**7**:e33218.
31. Zuko A, Kleijer KT, Oguro-Ando A, Kas MJ, van Daalen E, van der Zwaag B, et al. Contactins in the neurobiology of autism. *Eur J Pharmacol* 2013;**719**:63–74.
32. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. *Nat Rev Drug Discov* 2015;**14**:58–74.
33. Compton AG, Albrecht DE, Seto JT, Cooper ST, Ilkovski B, Jones KJ, et al. Mutations in contactin-1, a neural adhesion and neuromuscular junction protein, cause a familial form of lethal congenital myopathy. *Am J Hum Genet* 2008;**83**:714–724.
34. Hopkinson NS, Nickol AH, Payne J, Hawe E, Man WD, Moxham J, et al. Angiotensin converting enzyme genotype and strength in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;**170**:395–399.
35. Hopkinson NS, Eleftheriou KI, Payne J, Nickol AH, Hawe E, Moxham J, et al. +9/+9 Homozygosity of the bradykinin receptor gene polymorphism is associated with reduced fat-free mass in chronic obstructive pulmonary disease. *Am J Clin Nutr* 2006;**83**:912–917.
36. Broekhuizen R, Grimble RF, Howell WM, Shale DJ, Creutzberg EC, Wouters EF, et al. Pulmonary cachexia, systemic inflammatory profile, and the interleukin 1beta-511 single nucleotide polymorphism. *Am J Clin Nutr* 2005;**82**:1059–1064.
37. Hopkinson NS, Li KW, Kehoe A, Humphries SE, Roughton M, Moxham J, et al. Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease. *Am J Clin Nutr* 2008;**87**:385–390.
38. Wan ES, Cho MH, Boutaoui N, Klanderma B, Sylvia JS, Ziniti JP, et al. Genome-wide association analysis of body mass in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2011;**45**:304–310.
39. Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nat Genet* 2007;**39**:724–726.
40. Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD, et al. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. *PLoS One* 2007;**2**:e1361.
41. Hunt SC, Stone S, Xin Y, Scherer CA, Magness CL, Iadonato SP, et al. Association of the FTO gene with BMI. *Obesity (Silver Spring)* 2008;**16**:902–904.
42. Price RA, Li WD, Zhao H. FTO gene SNPs associated with extreme obesity in cases, controls and extremely discordant sister pairs. *BMC Med Genet* 2008;**9**:4.
43. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007;**3**:e115.
44. Albuquerque, D., Nobrega, C. & Manco, L. Association of FTO polymorphisms with obesity and obesity-related outcomes in Portuguese children. *PLoS One* **8**, e54370.
45. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 2007;**316**:889–894.
46. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2015. *J Cachexia Sarcopenia Muscle* 2015;**6**:315–316.