Research Article

Altered Gene Expression Profiles in Peripheral Blood Mononuclear Cells in Obese Subjects

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Abstract

Introduction: Gene expression profiles in human peripheral blood mononuclear cells (PBMCs) may act as a useful tool to better understand obesity. We investigated gene expression levels in PMBCs for possible differences between obese and non-obese subjects (19–55 years) and evaluated correlations between gene expression in PBMCs and clinical obesity indices. ***Methods:*** Body weight, BMI, fat amount, fat percentage, waist/hip ratio, leptin, and adiponectin levels were determined in 30 obese and 20 non-obese subjects. Expression levels of 19 genes, which were differentially expressed by clinical obesity indices in the PBMCs of high fat-fed rats, were determined in their PBMCs using real-time PCR. ***Results:*** The expression of 9 of 19 previously selected genes was significantly correlated with one or more clinical obesity indices. Both TFEC and CCL2 expression were negatively correlated with BMI, fat amount, fat percentage, waist/hip ratio, and leptin concentration. Similarly, TNFAIP2, VCAN, ASSI, IRF1, and HK3 expression negatively correlated with some clinical obesity indices, such as TNFAIP2 for BMI, fat amount, fat percentage, and waist/hip ratio, VCAN for fat amount, fat percentage, and waist/hip ratio, ASS1 for BMI and fat amount, IRF1 for BMI, fat amount, and fat percentage, and HK3 for fat amount. In contrast, both TNF-α and LPL expression were positively cor-

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related with waist/hip ratio. **Conclusion:** We identified 9 of 19 genes in human PBMCs that significantly correlated with one or more clinical obesity indices. Because these genes have a mechanistic basis for the development or progression of obesity and its metabolic derangements, they may help to determine possible underlying mechanisms for obesity.

Introduction

Overweight and obesity are abnormal and excessive fat accumulation, each of which is diagnosed when body mass index (BMI) is ≥ 25 and ≥ 30 kg/m^2 for adults, respectively [1]. The World Health Organization (WHO) has lowered the cutoff BMI for obese Asians to >27.5 kg/m^2 [2]. Overweight and obesity are major risk factors for a number of chronic diseases such as diabetes, cardiovascular diseases, and cancer. Obesity is more troublesome in children than in adults because overweight and obese children are likely to stay obese during their entire lifetime, rendering them at a higher risk for various metabolic diseases even at young adulthood [3]. Therefore, early identification of individuals who are overweight, or likely to develop overt obesity at a later stage of life, is important.

A biomarker is an indicator that objectively measures and evaluates the normal or pathogenic biological process [4]. Biomarkers can be used to screen and diagnose a disease. Biomarkers are also helpful to evaluate the effect of a drug and to predict the prognosis of a disease. Because blood interacts with every tissue and organ in the body via the circulation, blood-derived biomarkers may reflect general health or the disease status. For example, hemoglobin A$_{1c}$ in blood is a well-established biomarker or surrogate for diabetes that indicates the presence and severity of hyperglycemia. However, no novel blood-derived biomarker is currently available to diagnose subclinical obesity, to predict the prognosis of obesity, or to evaluate the effect of an anti-obesity treatment.

Recent studies showed that gene expression profiles assessed in peripheral blood mononuclear cells (PBMCs) may act as biomarkers for several diseases, such as Behçet's disease [5], Crohn's disease [6], and ulcerative colitis [6]. PBMCs are mainly composed of monocytes and lymphocytes, which undergo immunophenotypic changes in various diseases [7]. The usefulness of PBMCs as a source of early biomarkers for obesity was tested in a previous study, where altered expression of key energy homeostatic genes in PBMCs signaled metabolic imbalances that could lead to obesity [8]. However, there is still insufficient evidence demonstrating the utility of gene expression profiles in human PBMCs to detect subclinical obesity or overweight.

The objectives of the present study were to evaluate and compare several gene expression profiles in human PBMCs between obese and non-obese subjects, and to identify the correlations between PBMC-derived gene profiles and clinical obesity indices.

Materials and Methods

Subjects and Clinical Study
Males or females 19–55 years of age with a BMI <27 kg/m^2 (non-obese group) or ≥ 27 kg/m^2 (obese group), who were generally healthy otherwise based on a detailed medical history, physical examination, and laboratory tests, were included in this study. Subjects were excluded if they had taken any prescription medication for weight loss within 4 weeks prior to the time of blood sampling for PBMCs or had a history of changes in body weight $>10\%$ within 6 months. All subjects visited the Clinical Trials Center at the Seoul National University

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Table 1. Primer sequences used for real-time PCR analyses

Gene description (symbol)	Forward primer (5'–3')	Reverse primer (5'–3')
Chemokine (C-X-C motif) ligand 11 (CXCL10)	GGAATCTTTCTGCTTTGGGG	GGCAGTGGAAGTCCATGAAG
Ubiquitin D (UBD)	TGCAGGACCAGGTTCTTTTG	AGCTCCTCATCACGGGCTT
Immunoresponsive gene 1(IRG1)	CTGGCATTCATGGAATGAGG	TCCAGTTTCGGGAGCATGAAG
Hexokinase 3 (HK3)	GTGGACTTCCAGCAGAAGCA	AACCCTTGGTCCAGTTCAGG
Chemokine (C-X-C motif) ligand 11 (CXCL11)	GTCCATGGAATCCTGAACCC	GCAGCAAGTGTCCCAAAGAA
Argininosuccinate synthetase 1 (ASS1)	CTCCACGATGTCAATACGGC	CAACACCCCTGACATTCTCG
Serine (or cysteine) peptidase inhibitor, clade G, member 1 (SERPING1)	TCTCCATGATGGCCTTGAAA	GAAGTACCCTGTGGCCCATT
Tumor necrosis factor-α-induced protein 2 (TNFAIP2)	CCAGACAAGCCTAGGCAACA	GTCATGGCATCAAGGCTCAC
Transcription factor EC (TFEC)	AGAGAGCCCGAGAATTGGAA	AATCAACCGTGCCAAGTGAA
Interleukin 1β (IL-1β)	TGAAGCCCTTGCTGTAGTGG	ACAGGCTGCTCTGGGATTCT
Interferon regulatory factor 1 (IRF1)	GGTGATGTGGCATTTTCTGC	TACAAAAGCAGCCCGCTCTA
Signal transducer and activator of transcription 1 (STAT1)	ATTGCGAATGATGTCAGGGA	GCCATCACATTCACATGGGT
Versican (VCAN)	CAGTCCAACGGAAGTCATGC	CCCATCTCACAAGCATCCTG
Lipoprotein lipase (LPL)	TCAACATGCCCAACTGGTTT	AGTATGCAGAAGCCCCGAGT
Toll-like receptor 2 (TLR2)	ATGTTCCTGCTGGGAGCTTT	ACTGGTGTCTGGCATGTGCT
Toll-like receptor 4 (TLR4)	AATGCCCACCTGGAAGACTC	CCACATGTCAGGCCTTATGC
Chemokine (C-C motif) ligand 2 (CCL2)	CTTCGGAGTTTGGGTTTGCT	GCCAAGGAGATCTGTGCTGA
Interleukin 6 (IL-6)	CAGCTCTGGCTTGTTCCTCA	TGGCTGAAAAAGATGGATGC
Tumor necrosis factor-α (TNF-α)	CGGATCATGCTTTCAGTGCT	TGAAAACAACCCTCAGACGC

Hospital after a 12-h overnight fast and provided written informed consent and necessary information. Thereafter, their body weight, height, BMI, fat, fat percentage, and waist/hip ratio were determined using InBody 570® (InBody, USA). The subjects were divided into 2 groups according to their BMI. On the same day, blood samples were collected after a 12-h overnight fast to determine the gene profiles in PBMCs, leptin, adiponectin, insulin, total cholesterol, triglycerides, and free fatty acids.

PBMC Isolation

PBMCs were isolated by passing blood samples through Ficoll-Paque (GE Healthcare) at 400 *g* for 30 min. Following buffy coat removal from the plasma-Ficoll interface, cells were washed once in phosphate-buffered saline before being frozen for use at a later time.

RNA Extraction and Real-Time PCR

We previously identified 19 genes, which were differentially expressed by clinical obesity indices in the PBMCs of high fat-fed rats using the whole genome microarray analysis method [9]. Those PMBC genes included several genes with known biological functions related to obesity and metabolic syndrome, for example, C-X-C motif chemokine ligand 10 (CXCL10, +49.0-fold) [10], ubiquitin D (UBD, +9.9-fold) [11], immunoresponsive gene 1 (IRG1, +8.7-fold) [12], hexokinase 3 (HK3, +8.3-fold) [13], C-X-C motif chemokine ligand 11 (CXCL11, +7.1-fold) [14], argininosuccinate synthetase 1 (ASS1, +6.2-fold) [15], serine (or cysteine) peptidase inhibitor, clade G, member 1 (SERPING1, +5.4-fold) [16], tumor necrosis factor-α-induced protein 2 (TNFAIP2, +4.9-fold) [17], transcription factor EC (TFEC, +4.6-fold) [18], interleukin 1β (IL-1β, +4.5-fold) [19], interferon regulatory factor 1 (IRF1, +4.4-fold) [20], signal transducer and activator of transcription 1 (STAT1, +3.0-fold) [21], versican (VCAN, +2.9-fold) [22], lipoprotein lipase (LPL, +2.9-fold) [23], toll-like receptor 2 (TLR2, +2.9-fold) [24], toll-like receptor 4 (TLR4, +2.9-fold) [24], C-C motif chemokine ligand 2 (CCL2, +2.7-fold) [25], interleukin 6 (IL-6, +1.8-fold) [26], and tumor necrosis factor-α (TNF-α, +1.5-fold) [26].

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Table 2. Baseline demographic and biochemical characteristics of obese (n = 30) and non-obese (n = 20) adults

	Obese (n = 30) (means ± SD)	Non-obese (n = 20) (means ± SD)	p value
Age, years	30.77±8.97	31.10±9.49	0.9003
Females/males	12/18	0/20	
Weight, kg	84.11±9.63	67.74±10.15	<0.0001
Height, cm	169.57±8.39	172.46±5.46	0.1802
BMI, kg/m^2	29.36±2.30	22.73±2.85	<0.0001
Fat, kg	30.14±5.89	13.82±6.86	<0.0001
Fat, %	35.93±7.02	19.87±7.73	<0.0001
Waist/hip ratio	0.96±0.05	0.84±0.05	<0.0001
Leptin, pg/mL	14,528.27±12,166.70	1,985.20±1,858.01	<0.0001
Adiponectin, ng/mL	3,953.47±1,652.06	6,512.60±3,496.73	0.0053
Insulin, mU/L	14.69±7.25	21.42±19.76	0.1582
Total cholesterol, mg/dL	188.77±34.60	178.50±22.41	0.2473
Triglycerides, mg/dL	129.70±64.30	119.65±60.20	0.5814
Free fatty acids, uEq/L	478.00±156.64	555.90±356.71	0.3671

All subjects were in the fasting state prior to blood sampling and measurements of weight, height, BMI, fat amount, fat percentage, and waist/hip ratio. Student's t test was applied.

Total RNA was extracted from PBMC using the TRIzol reagent (Life Technology, Carlsbad, CA, USA) according to the manufacturer's instructions. cDNA synthesis was performed with 1 µg of total RNA, 5× RT buffer, 2.5 mM each dNTP, 0.1 M dithiothreitol, 200 U/µL reverse transcriptase, and 40 U/µL RNase inhibitor at 37°C for 2 h. Quantitative PCR was performed (after RNA reverse transcription) using iQ SYBR Green supermix (Bio-Rad, Hercules, CA, USA) with the CFX Connect™ real-time PCR detection system (Bio-Rad). All gene expression data were normalized to GAPDH. Primer sequences are listed in Table 1. The results on the optical density ratio of a target gene to GAPDH are presented as means ± SEM of at least 3 different experiments.

Statistical Analysis

All data were expressed as means ± SD. The differences in gene expression profiles and clinical variables between the obese and the non-obese group were analyzed using the two-sample Student's t test after log transformation when deemed appropriate. Spearman's correlation coefficient was also used to evaluate the association between the obesity parameters and gene expression profiles in PBMCs. A two-tailed $p < 0.05$ was considered statistically significant.

Results

Subjects

A total of 30 obese (12 females and 18 males) and 20 non-obese subjects (no female and 20 males) were included in the study. Because the subjects were enrolled in the order of providing written informed consent, there were no female subjects in the non-obese group. Age, height, and baseline levels of insulin, total cholesterol, triglycerides, and free fatty acids did not differ between the obese and non-obese groups, whereas body weight, BMI, fat amount, fat percentage, waist/hip ratio, and baseline level of leptin were significantly higher

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Table 3. Gene expression levels of 19 selected genes, which were differently expressed between obese and non-obese rats, in obese (n = 30) and non-obese (n = 20) adults

Gene name	Obese (n = 30) (means ± SD)	Non-obese (n = 20) (means ± SD)	p value
TFEC	0.41±0.36	0.75±0.47	0.0052
CCL2	13.99±9.98	25.44±14.00	0.0014
TNFAIP2	43.07±32.67	58.23±22.33	0.0766
VCAN	29.51±16.21	36.24±17.02	0.1648
ASS1	7.83±4.05	11.41±4.52	0.0052
HK3	2.38±2.10	2.64±0.88	0.5554
TNF-α	13.25±27.38	3.76±2.02	0.0686
IL-1β	10.10±17.02	1.59±0.99	0.0105
LPL	13.69±15.62	8.12±5.90	0.0839
IRF1	16.34±13.14	17.68±6.57	0.6352
STAT	16.15±10.60	17.47±8.79	0.6481
IRG1	6.51±7.33	5.77±4.25	0.6528
TLR2	8.34±7.68	12.32±16.93	0.3339
SERPING1	12.91±9.83	17.22±23.15	0.4399
UBD	29.79±20.00	22.18±12.45	0.1038
CXCL10	5.11±10.91	2.88±3.06	0.2959
TLR4	27.07±52.19	16.31±7.54	0.2746
IL-6	478.52±2573.30	7.49±4.29	0.3244
CXCL11	22.03±42.62	13.92±12.43	0.3329

These 19 genes were selected for analysis based on previous animal experimental data. TFEC, transcription factor EC; CCL2, chemokine (C-C motif) ligand 2; TNFAIP2, tumor necrosis factor-α-induced protein 2; VCAN, versican; ASS1, argininosuccinate synthetase 1; HK3, hexokinase 3; TNF-α, tumor necrosis factor-α; IL-1β, interleukin 1β; LPL, lipoprotein lipase; IRF1, interferon regulatory factor 1; STAT, signal transducer and activator of transcription 1; IRG1, immunoresponsive gene 1; TLR2, toll-like receptor 2; SERPING1, serine peptidase inhibitor, clade G, member 1; UBD, ubiquitin D; CXCL10, chemokine (C-X-C motif) ligand 10; TLR4, toll-like receptor 4; IL-6, interleukin 6; CXCL11, chemokine (C-X-C motif) ligand 11. Student's t test was employed.

in the obese group than in the non-obese group (Table 2). Furthermore, baseline adiponectin concentration was significantly lower in the obese group than the non-obese group (3,953.47 vs. 6,512.60 ng/mL, p = 0.005; Table 2).

Gene Expression in PBMCs

Gene expression levels of a total of 19 previously selected genes were compared between obese and non-obese subjects. IL-1β gene expression in PBMCs was significantly higher in the obese subjects than in the non-obese subjects (10.10 vs. 1.59, p = 0.011; Table 3). Likewise, IL-6 and TNF-α gene expression in PBMCs was higher in the obese individuals than in the non-obese controls although differences were not statistically significant. In contrast, TFEC, CCL2, and ASS1 gene expression was significantly lower in the obese subjects than in the non-obese controls (0.41 vs. 0.75; 13.99 vs. 25.44; and 7.83 vs. 11.41, respectively; Table 3). Other gene expression levels were not significantly different between the obese and the non-obese group.

Correlations between Gene Expression in PBMCs and Clinical Obesity Indices

Nine out of the 19 human PBMC genes had a statistically significant correlation with one or more of the clinical obesity indices (Fig. 1, 2; Table 4). TFEC, CCL2, TNFAIP2, VCAN, ASSI, IRF1, and HK3 showed a negative correlation with BMI, fat amount, fat percentage, waist/hip ratio, and leptin, many of which were statistically significant. In contrast, TNF-α and LPL gene
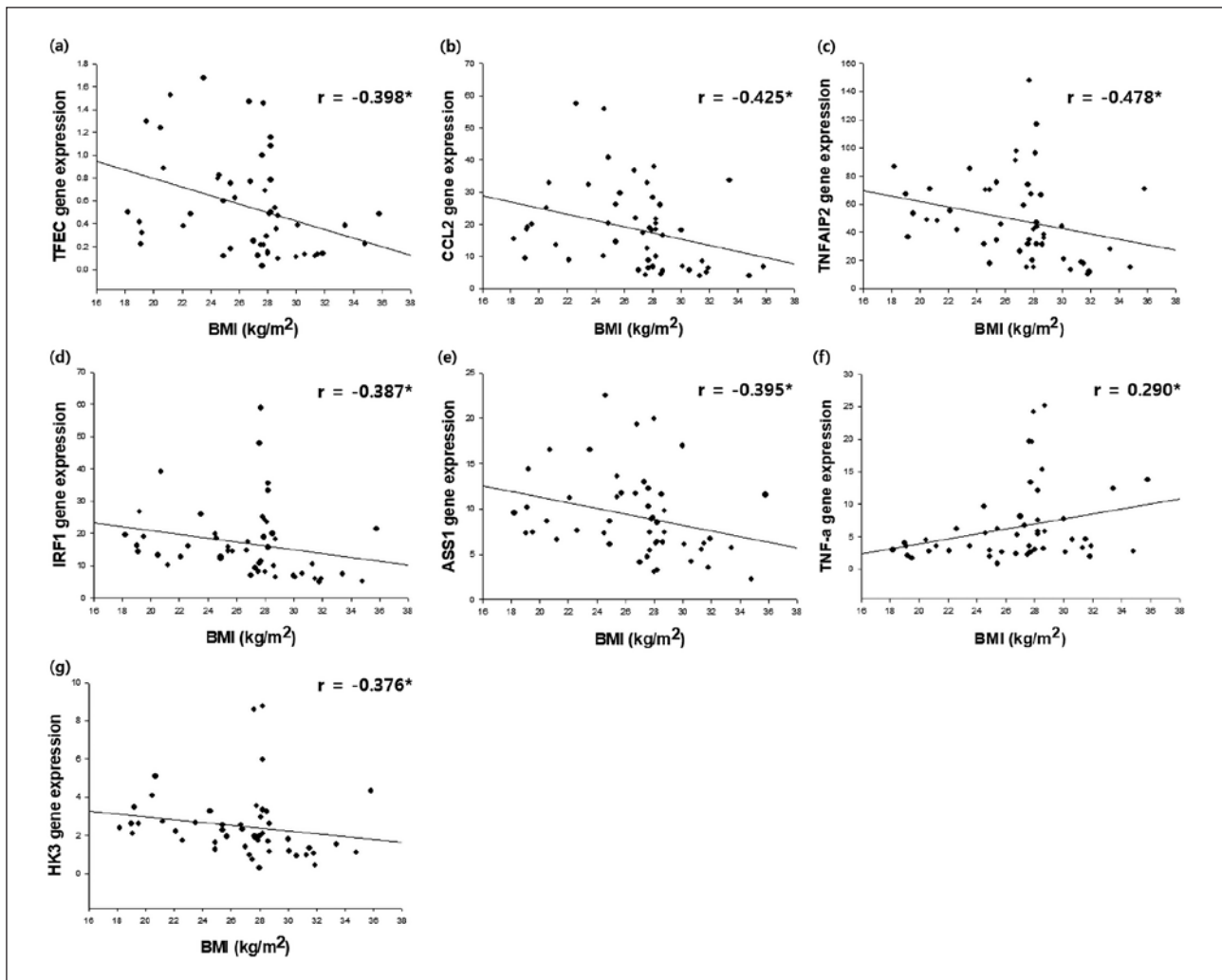
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Fig. 1. Correlation of gene expression of TFEC (**a**), CCL2 (**b**), TNFAIP2 (**c**), IRF1 (**d**), ASS1 (**e**), TNF-α (**f**), and HK3 (**g**) with BMI in adults ($n = 50$: 30 obese and 20 non-obese adults). * $p < 0.05$. TFEC, transcription factor EC; CCL2, chemokine (C-C motif) ligand 2; TNFAIP2, tumor necrosis factor-α-induced protein 2; IRF1, interferon regulatory factor 1; ASS1, argininosuccinate synthetase 1; TNF-α, tumor necrosis factor-α; HK3, hexokinase 3.

expression levels in PBMCs were positively correlated with BMI, fat amount, fat percentage, waist/hip ratio, and leptin concentration, of which the correlations of BMI, waist/hip ratio, and leptin concentration with TNF-α were statistically significant, and the correlation of waist/hip ratio and LPL was statistically significant. On the other hand, adiponectin had a statistically significant negative correlation with TNF-α ($r = -0.307$, $p = 0.032$; Table 4) although it did not show a noticeable correlation with the expression of other genes in PBMCs.

Discussion

We previously screened a total of 19 candidate murine PBMC genes for obesity in high fat-fed rats [9]. Similar to that study, we used microarray gene expression patterns in the present study to investigate differences in the expression profiles of those 19 genes in human
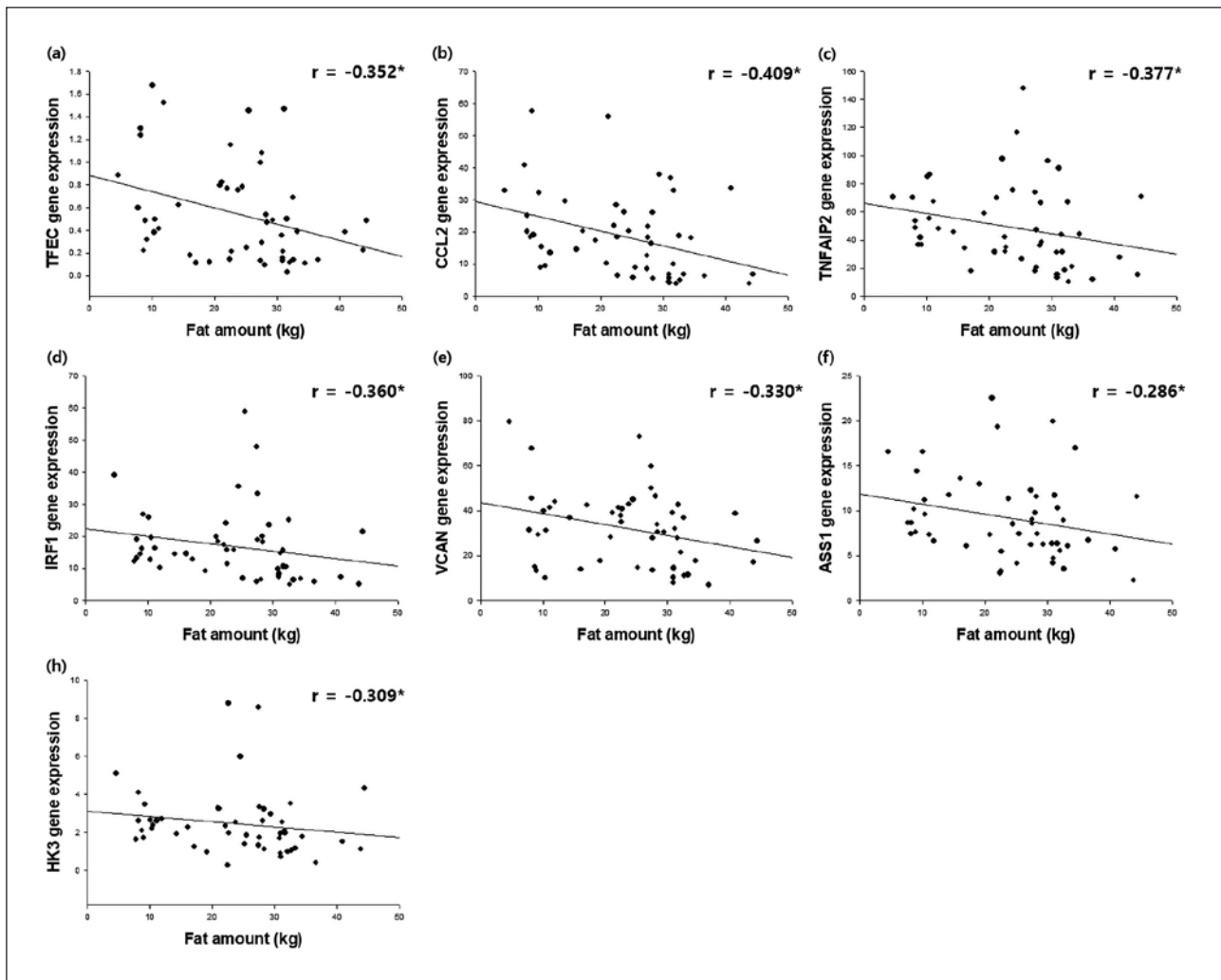
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Fig. 2. Correlation of gene expression of TFEC (**a**), CCL2 (**b**), TNFAIP2 (**c**), IRF1 (**d**), VCAN (**e**), ASS1 (**f**), and HK3 (**g**) with fat amount in adults ($n = 50$: 30 obese and 20 non-obese adults). * $p < 0.05$. TFEC, transcription factor EC; CCL2, chemokine (C-C motif) ligand 2; TNFAIP2, tumor necrosis factor-α-induced protein 2; IRF1, interferon regulatory factor 1; VCAN, versican; ASS1, argininosuccinate synthetase 1; HK3, hexokinase 3.

PBMCs between obese and non-obese subjects. As a result, 9 of those 19 candidate genes in human PBMCs were significantly correlated with one or more clinical obesity indices such as BMI, fat amount, fat percentage, waist/hip ratio, leptin, and adiponectin in this study.

ASS1, CCL2, and TFEC were not only significantly lower in the obese subjects than in the non-obese controls, they were also negatively correlated with clinical obesity indices, nearly all of which were statistically significant. ASS1 is the enzyme responsible for citrulline metabolism in mammals [27], and argininosuccinate produced via ASS1 is the immediate precursor of arginine, which leads to the production of nitric oxide (NO) in many cells [28]. The availability of NO was diminished in the obese state [29], and low levels of NO increased inflammatory response in vessel lumens [30]. Decreased ASS1 expression in obese subjects reduces NO production, which could contribute to the development of atherosclerosis in this population. Therefore, underexpressed ASS1 can be a potential biomarker for obesity as shown in the present study.

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Table 4. Spearman's correlation coefficients between 9 genes, which were differently expressed between obese and non-obese adults, and obesity parameters

Gene	BMI, kg/m^2	Fat, kg	Fat, %	Waist/ hip ratio	Leptin, pg/mL	Adiponectin, ng/mL	Insulin, mU/L	Total C, mg/dL	Triglycer- ide, mg/dL	FFA, µEq/L
TFEC	–0.398*	–0.352*	–0.315*	–0.337*	–0.35*	–0.038	–0.063	–0.097	0.049	0.113
CCL2	–0.425*	–0.409*	–0.338*	–0.352*	–0.358*	0.09	–0.198	–0.064	0.093	0.137
TNFAIP2	–0.478*	–0.377*	–0.316*	–0.335*	–0.28	0.069	0.018	–0.167	–0.117	–0.065
IRF1	–0.387*	–0.360*	–0.286*	–0.238	–0.286*	–0.089	–0.076	–0.243	–0.066	0.087
VCAN	–0.263	–0.330*	–0.290*	–0.290*	–0.165	–0.137	0.223	–0.005	0.088	0.11
ASSI	–0.395*	–0.286*	–0.211	–0.245	–0.243	0.07	–0.23	–0.191	–0.265	–0.085
TNF-α	0.290*	0.235	0.250	0.438*	0.28*	–0.307*	–0.054	0.241	0.26	0.107
HK3	–0.376*	–0.309*	–0.214	–0.233	–0.247	0.077	–0.018	–0.145	–0.036	0.106
LPL	0.116	0.159	0.168	0.339*	0.185	–0.236	0.014	0.086	–0.098	–0.131

C, cholesterol; FFA, free fatty acids; TFEC, transcription factor EC; CCL2, chemokine (C-C motif) ligand 2; TNFAIP2, tumor necrosis factor-α-induced protein 2; IRF1, interferon regulatory factor 1; VCAN, versican; ASS1, argininosuccinate synthetase 1; TNF-α, tumor necrosis factor-α; HK3, hexokinase 3; LPL, lipoprotein lipase. * $p < 0.05$.

CCL2 and TFEC gene expression is more complicated, and inconsistent findings have been reported. The CCL2 (also known as MCP-1) gene is a major chemokine that regulates migration and infiltration of monocytes and macrophages. CCL2 expression in human adipose tissue was positively correlated with the degree of obesity [31], and the plasma level of CCL2 was increased in obese adults compared with normal weight subjects [25, 32]. Likewise, TFEC is a known mediator of proinflammatory G-CSF (granulocyte colony-stimulating factor) gene expression, and TFEC was associated with insulin resistance in human adipocytes [33]. However, other studies showed conflicting results in agreement with ours. For example, expression of proinflammatory genes including IL-6, IL-1β, IL-1Ra, and CCL2 in PBMCs was reduced in the obese group [34, 35]. Therefore, reduced expression of the CCL2 and TFEC genes in PBMCs in our results may represent a compensatory response to maintain homeostasis. Namely, proinflammatory gene expression in PBMCs could have been suppressed to maintain the body homeostasis in the low-grade inflammatory state caused by obesity.

The same homeostasis hypothesis may explain why the expression levels of the VCAN and TNFAIP2 genes in PBMCs were reduced in obese subjects in our results. VCAN plays an important role in lipid accumulation, inflammation, and thrombosis [22]. Furthermore, TNFAIP2 significantly increased inflammatory response with TNF-α [36]. Therefore, VCAN and TNFAIP2 expression is likely to be increased in obese individuals. However, the opposite finding was observed in the present study, which could again be explained by the homeostasis hypothesis.

The differences in TNF-α and LPL expression in PBMCs between the obese and the non-obese subjects was similar to those seen in previous studies. For example, the expression of TNF-α in PBMCs increased with increasing obesity. The mRNA level of TNF-α was significantly increased as leptin concentration was increased [37]. Furthermore, overexpression of TNF-α induced by leptin is more sensitive in obese subjects than in normal-weight individuals. On the other hand, clinical obesity indices were positively correlated with LPL expression in the liver and muscle tissue [38]. This suggests that LPL expression was increased in endothelial cells located in the liver and muscle tissue, and triglycerides migrated from lipoprotein to the tissue in obese patients [38].

The expression of HK3 and IRF1 in PBMCs tended to decrease in the obese subjects compared with the non-obese controls although they failed to show statistical significance. HK3 expression was inhibited by physiological concentration of glucose [39]. Therefore,

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increased blood glucose concentration in obese patients could inhibit the action of HK3. Similarly, IRF1 has been reported to regulate adipogenesis, and reduced IRF1 could elevate lipid accumulation [40], which is likely to happen in obese subjects as reflected in our results.

The present study had several limitations. First, the number of obese subjects was small. Second, the mean BMI of obese subjects in this study was relatively modest, i.e., 29.36 kg/m^2 (range: 27.0–35.8 kg/m^2). Therefore, our results may differ from the findings in subjects with extreme or severe obesity. In this study, we used a BMI of 27 kg/m^2 as the cutoff that divided subjects into obese and non-obese subjects, consistent with the WHO classification in Asians. Thus, the obesity criteria adopted in the present study were sufficient to show the difference in the gene expression between the obese and the non-obese subjects. Third, we did not test if the identified gene expression could change in response to obesity treatment. Further studies with a larger number of obese subjects are warranted to confirm our findings.

Conclusion

We identified 9 genes out of the 19 candidate genes in human PBMCs that significantly correlated with one or more clinical obesity indices: ASS1, CCL2, TFEC, VCAN, TNFAIP2, TNF-α, LPL, HK3, and IRF1. These genes have a mechanistic basis for the development or progression of obesity and its metabolic derangements, which may help to determine possible underlying mechanisms for obesity.

Acknowledgment

Statement of Ethics

The study protocol was reviewed and approved by the Institutional Review Board of the Seoul National University Hospital (IRB No. H-1411-097-628). All the subjects provided written informed consent after a detailed explanation of the study prior to any study procedure. This study was conducted in full accordance with the principles of the Declaration of Helsinki and Good Clinical Practice as codified in the ICH guidelines (ICH E6) and other regulations.

Disclosure Statement

The authors have no conflicts of interest to declare.

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