Identification of Pathways Associated With Endothelial Dysfunction in Obesity

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ABSTRACT

Obesity has become an increasingly serious health problem and a widespread social focus. It is associated with many diseases, including cardiovascular disease through the induction of endothelial dysfunction. The aim of this study was to analyze the endothelial genes that become dysfunctional in obesity and then describe the most significant signal pathways using a systematic bioinformatics approach. Two sets of genes were recovered from the PubMed database. One set was constrained to vascular endothelial dysfunction (VED), and the other set was strictly constrained to both obesity and VED. These two gene sets were mapped to the pathway databases in GeneGo and DAVID to identify pathways associated with endothelial dysfunction in obesity. One hundred and fifty-nine genes were collected in the first set of VED genes. An additional 64 genes associated with obesity also played a role in endothelial dysfunction. Two major pathways (Proopiomelanocortin (POMC) processing and angiotensin system maturation in protein folding and maturation) associated with obesity and endothelial dysfunction were explored. This paper studied obesity at the systems biology level and identified important pathways associated with this disorder. These pathways could serve as targets for treatment and drug discovery in the future.

KEYWORDS
Pathway, endothelial dysfunction, obesity

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1. INTRODUCTION

Obesity is a disorder characterized by the excessive deposition of fat. In response to an increase in the incidence of childhood simple obesity (CSO), this disorder has attracted increasing attention. CSO is not only an endocrine disease but also a major risk factor for vascular endothelial dysfunction.

Vascular endothelial dysfunction (VED) is characterized by altered endothelium-mediated vasodilatation, increased vascular reactivity, platelet activation, thrombus formation, increased permeability, and leucocytes adhesion [1]. VED is an early pathological step in various diseases such as high blood pressure, coronary heart disease, heart failure, diabetes, obesity, and Kawasaki disease.

Genetic studies of CSO and vascular endothelial function were conducted not only to clarify the genetic background of these disorders but also in the hope of providing clues about etiology and pathogenesis. However, previous studies have mostly focused on one or two genes of obesity. For example, Foxo1 from the hypothalamus is an important regulator of food intake and energy balance [2]. Also, obesity causes an increase in the expression of the angiotensinogen gene [3], and α-MSH ameliorates endothelial dysfunction associated with diet-induced obesity [4]. POMC can reduce the morbidity rate of type 2 diabetes and obesity by regulating glucose metabolism [5] and influencing cardiac growth and renewal via daily rhythmicity [6]. Inactivation of the melanocortin-4 receptor (MC4-R) results in adult-onset obesity syndrome [7]. FTO has been associated with both adiposity and food intake [8]. The TMEM18 gene may be heavily involved in the modulation of energy homeostasis [8], and endothelial NO-synthase (eNOS), NADPH-oxidase, and methylenetetrahydrofolate reductase (mTHFR) are involved in the modulation of endothelial function by obesity [9].
Few studies have analyzed all of these genes systematically. In this pilot study, statistically significant associations between genes and vascular endothelial function and obesity were identified to analyze gene function (up to March 2013 from the PubMed database). This study is a systematic summary of previous research. The purpose was to identify the most relevant genes and pathways in obesity and vascular endothelial dysfunction and then report them in a further study after clinical verification.

2. METHODS AND MATERIALS

2.1. Extracting genes from PubMed

In this study, we selected two sets of genes. Group A contained genes relevant to vascular endothelial dysfunction, while Group B contained genes relevant to obesity characterized by vascular endothelial dysfunction. A computerized search of the PubMed database (http://www.ncbi.nlm.nih.gov/gene/) up to March 2013 was performed, and cited references were reviewed to identify relevant studies. Citations were screened at the title/abstract level and retrieved as full reports. Human genes with an effect on vascular endothelial function were included in this study. Group A genes were selected with the keywords “polymorphism”, “gene”, “genetic”, “allele”, “genotype”, “genetics” and “genome” in combination with “endothelial function” and “endothelium”. Group A genes, which were thought to be more relevant, were found in the conclusion of articles. Group B genes were identified with the keywords “polymorphism”, “gene”, “genetic”, “allele”, “genotype”, “genetics” and “genome” in combination with “endothelial function”, “endothelium”, “obesity”, “adiposis”, “adiposity”, and “fat”. Group B genes were chosen from the full text of the articles being searched.

2.2. Data analysis

GeneGo (http://www.genego.com, version: 6.5) and DAVID (the Database for Annotation, Visualization and Integrated Discovery, http://david.abcc.ncifcrf.gov/, version: 6.7) are biological information databases that provide comprehensive biological-function annotation and pathway-enrichment analysis. GeneGo MetaCore was used to analyze the pathways containing genes from this study. DAVID was used to process the bioinformatics analysis of these candidate gene markers, including gene biological processes (GOTERM_BP_FAT), functional categories (SP_PIR_KEYWORDS), diseases (OMIM_DISEASE), and pathways (KEGG_PATHWAY), among other categories.

3. RESULTS

3.1. Extracting genes

The characteristics of genes from groups A and B are presented in Tables 1 and 2 in supplementary materials, respectively.

3.2. Pathway enrichment analysis

Pathway enrichment analysis consisted of matching genes in functional ontologies using GeneGo Meta-Core. The probability of a random intersection between a set of genes and ontology entities was estimated with the “p” value of the hypergeometric intersection. A lower “p” value indicated a higher relevance of the entity to the dataset, which shows as a higher rating for the entity. All maps were drawn with GeneGo.

As is shown in Figure 1, the most significant GeneGo Pathway Maps in Group A gene were (1) immune response_CCL2 signaling, (2) the immune response_HMGB1/RAGE signaling pathway, (3) protein folding and maturation_angiotensin system maturation/human version and (4) immune response_IL-18 signaling. Other pathways were also identified, including NFAT signaling in cardiac hypertrophy, MIF-mediated glucocorticoid regulation, the HSP60 and HSP70/TLR signaling pathway, the IL-1 signaling pathway and MIF-induced cell adhesion, and migration and angiogenesis in the immune response. The most significant diseases included (1) coronary artery disease (2) wounds and injuries (3) myocardial infarction (4) and type 2 diabetes mellitus.
Most of the Group A genes played a role in pathways of immune response, protein folding and maturation, cardiac hypertrophy and diseases of cardiovascular disease and metabolic disease.

As is shown in Figure 2, the most significant GeneGo pathway maps in Group B were: (1) protein folding and maturation of POMC processing, (2) putative pathways for stimulation of fat-cell differentiation by bisphenol A, and (3) protein folding and maturation angiogenin system maturation human or rodent version. Other pathways were also identified, including the role of adiponectin in regulation of metabolism, MIF-mediated glucocorticoid regulation, and leptin signaling via the PI3K-dependent pathway. The most significant diseases were (1) obesity, (2) type 2 diabetes mellitus, (3) metabolic syndrome X, (4) insulin resistance, and (5) morbid obesity.

We concluded that most of the pathway maps in Group B were in the metabolic regulation of lipids and proteins, and the majority were involved in metabolic disease. Protein folding and maturation of POMC processing had a lower “p” value, indicating a higher relevance.

The pathway map protein folding and maturation angiogenin system maturation human version or rodent version appeared in both of the figures above. This pathway may be very important for vascular endothelial dysfunction caused by obesity.

### 3.3. Gene functional annotation analysis

Genes with statistical significance were subjected to functional annotation analysis using DAVID software. The GORTERM_BP_FAT, SP_PIR_KEYWORDS, OMIM_DISEASE and KEGG_PATHWAY analyses were based on p value, FDR and Enrichment Score. Group A and B genes can be found in DAVID and DAVID results (see supplementary materials), respectively.

For Group A, circulatory system processes (33 genes), blood circulation (33 genes), regulation of blood pressure (26 genes), vascular process in circulatory system (17 genes), regulation of blood vessel size (16 genes) and vasodilatation (six genes) were the most enriched (enrichment score=20.13). Furthermore, DAVID analysis identified clusters of genes with annotations related to the regulation of lipid metabolic process (enrichment score: 15.02); vasculature development, blood vessel morphogenesis, blood vessel development, and angiogenesis (enrichment score: 8.98); and regulation of apoptosis (enrichment score: 7.67). Additionally, the insulin signaling pathway (hsa04910), cytokine-cytokine receptor interaction (hsa04060), and hypertrophic cardiomyopathy (hsa05410) as a KEGG pathway were identified. There were also some diseases enriched, including myocardial infarction, coronary artery disease, and those related to metabolic syndrome pathways.

For Group B, GORTERM_BP_FAT analysis identified several significant biological processes, including brown-fat-cell and fat-cell differentiation (enrichment score: 8.11); lipid oxidation, fatty-acid oxidation, and lipid modification (enrichment score: 4.10); and the regulation of blood pressure, the regulation of foam cell differentiation, circulatory system processes, blood circulation, regulation of cellular ketone metabolic processes, regulation of lipid metabolic process, and lipid localization (enrichment score: 4.1). SP_PIR_KEYWORDS analysis identified functional categories significant in obesity (p value: 1.36E-18), diabetes mellitus (p value: 4.57E-08), and so on. OMIM_DISEASE analysis and genome-wide association studies yielded new sequence variants at seven loci that associated with measures of obesity (p value: 1.20E-13) and six new loci associated with the body mass index, highlighting a neuronal influence on body weight regulation (p value: 8.15E-12).

### 4. DISCUSSION

From these results, two pathway maps were shown to be very important for vascular endothelial dysfunction and obesity: POMC processing and angiogenin system maturation.
4.1. Protein folding and maturation of POMC processing pathway

Figure 3 shows the specific process of POMC processing. The POMC gene produces a 32 kDa propeptide via Carboxypeptidase H that is processed into regulated secretory granules. POMC is post-translationally cleaved within these granules by the serine proteases PC1 and PC2 (SPC2). PC1 cleaves POMC into β-LPH and proACTH. β-LPH is cleaved by PC1 and PC2 (SPC2) into γ-LPH and β-Endorphin in the extracellular region. Subsequently, gamma-LPH is cleaved by PC2 (SPC2) into β-MSH. PC1 and PC2 (SPC2) cleave proACTH to ACTH, N-POC, N-POMC and joining peptide (JP) and also ACTH to CLIP and ACTH 1-17. PC2 (SPC2) also cleaves N-POC to γ-MSH, γ2-MSH and γ3-MSH. PAM amidates ACTH 1-17 resulting in DA-γMSH, which is then acetylated by NAT-1 to become α-MSH.

POMC is present in various places, including pituitary, hypothalamus, central nervous system and skin tissues. POMC undergoes extensive post-translational processing by PC1 and PC2 (SPC2), resulting in the production of β-Endorphin, β-MSH, α-MSH and γ-MSH.

POMC is primarily expressed in the hypothalamus and pituitary gland. Studies in the early 90s found that the processing of POMC has tissue in addition to species specificity. For example, adrenocorticotropic hormone (ACTH) cells in the pituitary process POMC into ACTH and β-Lipotropin (β-LPH), and POMC from the center of the pituitary is processed into α-melanin (α-MSH), corticotropin-like intermediate peptide (CLIP), β-LPH, and β-endorphins. POMC induces early-onset obesity, or adolescent obesity, through its effects on feeding [151]. Mutations in this gene have been associated with early-onset obesity and type 2 diabetes [5].

Hypothalamic neurons, which are the main centers for the adjustment of food intake, include the arcuate nucleus (Arc) among others. POMC and agouti-related protein (AgRP)/neuropeptide Y (NPY)-expressing neurons are the most extensively studied neuronal populations in the Arc. Membrane depolarization of POMC neurons, which leads to α-MSH release and MC4R activation, ultimately decreases the desire for eating and thus reduces obesity. On the other hand, NPY released by AgRP/NPY neurons has the opposite effect, i.e., the boosting of appetite, and is mediated by different subtypes of NPY receptors on downstream neurons. AgRP directly blocks α-MSH mediated activation of MC4R, and is likely the result of NPY in food intake [152]. So the proportion of POMC and AgRP/NPY leads to different biological effects that are important in the regulation of food intake and energy homeostasis.

Both POMC and NPY/AgRP neurons are regulated by peripheral hormones such as leptin and insulin and also by nutrients such as fatty acids, amino acids and glucose. Insulin is secreted by pancreatic β cells in response to the blood glucose concentration in the body. Leptin is also secreted by adipocytes, and therefore circulating leptin concentrations increase with fat gain. Leptin promotes POMC and α-MSH secretion, and thus leptin can suppress appetite and prevent obesity. POMC is sensitive to glucose, which is necessary for the maintenance of normal glucose homeostasis and body weight. Signal transducer and activator of transcription 3 (STAT3) binding to the POMC promoter increases POMC mRNA expression by recruiting histone acetylases, whereas STAT3 in AgRP neurons decreases AgRP (and possibly NPY) expression by recruiting histone deacetylases [2, 153]. An increase in leptin could mask the full phenotype resulting from the lack of STAT3 signaling in POMC neurons by acting on neuron populations other than POMC neurons as well as by activating other leptin sensitive signaling pathways such as phosphatidylinositol 3-kinase (PI3K) signaling in POMC cells. PI3K mediates phosphorylation of FOXO1, which activates AgRP and inhibits POMC [153, 154].

Furthermore, the melanin cortisol receptor system (MCR) plays an integral role in regulating feeding. MCR has two kinds of ligands: activated and inhibitory. The former, composed of POMC shear, includes α-MSH, β-MSH, γ-MSH and adrenocorticotropic hormone (ACTH). The latter includes NPY and AgRP. Activity-dependent release of α-MSH from POMC neurons activates MC3Rs and MC4Rs and results in a potent inhibition of food intake [155]. However, mice deficient for MC4Rs are fatter than mice deficient for MC3Rs [7]. MSH improves endothelial function via the augmentation of NO availability [4]. During adenohypophysis, which is activated by corticotropin-releasing hormone (CRH), POMC decomposes...
into ACTH, beta-LPH and a small amount of amine. Just like POMC, CRH reduces fat synthesis by reducing energy intake and increasing energy consumption.

POMC exhibits rhythmic gene cycling similar to what is observed in the heart. In mice, POMC expression peaks after 4 hours of darkness at night and is lowest at 4–7 hours after lights are turned on in the day. Meanwhile, compared to the normal heart, POMC in cardiac hypertrophy reached a peak 8 hours after lights are turned off. POMC increases the heart rate and blood pressure in the subjective day or active period, and decreases them during the night or sleep period [6].

NPY may have both vasoconstrictive and vasodilatory activity. A leucine7 to proline7 substitution (Leu7Pro) in NPY is associated with enhanced endothelial-dependent vasodilation [106]. POMC inhibits NPY, so it takes part in vasoconstriction and the terminal effects of cardiovascular disease.

The POMC pathway has a significant role in the regulation of obesity and, in particular, early-onset obesity. The POMC protein folding, maturation and processing pathway produces many downstream products (LPH, MSH, ACTH, JP, and CLIP). All of these products participate in energy regulation. POMC also plays a role in the cardiovascular system. This pathway may affect the occurrence and development of vascular endothelial dysfunction resulting from obesity.

4.2. Protein folding and maturation—angiotensin system maturation

The pathway of protein folding and maturation—angiotensin system maturation for both Group A and Group B genes was significantly enriched, and is strongly correlated with vessel endothelium dysfunction in obesity.

Figure 4 shows the basic pathway of angiotensinogen maturation. A group of proteases hydrolyze angiotensinogen to angiotensin I. Angiotensin I in turn is hydrolyzed to produce angiotensin II by angiotensin I converting enzyme (ACE) among others. Angiotensin II is processed into angiotensin III and then angiotensin IV. Of these products, the effects of angiotensin II and III are the strongest, although the concentration of angiotensin III is lower. Therefore, angiotensin II plays a primary role in the renin-angiotensin system (RAS). This pathway participates in vasoconstriction, oxidative stress, sympathetic activation, sodium and water retention, vasodilation and cardiac hypertrophy, among other processes. It is a key pathway related to cardiovascular disease.

Many studies have shown that RAS is associated with obesity, and the inhibition of RAS may have a beneficial effect in controlling this disorder. The expression of angiotensinogen (AGT) in diet-induced obese mice has tissue specificity. AGT is significantly elevated in intra-abdominal fat but not in other fat depots or nonadipose tissues[3]. Therefore, obesity driven by diet is characterized primarily by abdominal obesity.

Angiotensin II plays the most important role in RAS and ultimately alters cardiovascular function and structure through a variety of mechanisms. The primary functions of angiotensin II are as follows. (1) Angiotensin II can directly promote arteriole contraction and elevate blood pressure as well as promote venous contraction and increase venous return. (2) Angiotensin II can be applied to the presynaptic angiotensin II receptor in the sympathetic shrinkage of fiber peripheral vessels and multiply neurotransmitters released from the sympathetic nerve endings. (3) Angiotensin II increases peripheral vascular resistance and blood pressure. (4) Angiotensin II strongly stimulates adrenal-cortex zona cells to synthesize and release aldosterone and then promotes renal tubular reabsorption of Na+.; (5) Angiotensin II promotes the degradation of bradykinin, which releases the vasodilator nitric oxide[156]. (6) Angiotensin II promotes oxidative stress by increasing the production of oxygen-based free radicals [157].

Many previous studies have elucidated the relationship between RAS and cardiovascular disease. (1) For hypertension, angiotensin II can activate endothelial NADH/NADPH oxidase and induce production of super oxygen anions. Angiogensin II can also shrinks blood vessels and reduces the vasodilating properties of NO. Angiotensin II and prostaglandin can stimulate the release of endothelin, causing vascular smooth muscle contraction, endothelial damage, smooth-muscle-cell proliferation, and vascular wall remodeling [158, 159]. (2) Angiotensin converting enzyme (ACE), AGT and Angiotensin II
are significantly elevated in the myocardium of patients with chronic congestive heart failure [160]. (3) For atherosclerosis, monocytes and macrophages aggregate on vessel walls, following binding between angiotensin II and the AT1 receptor, to form terminal foam cells by absorbing oxLDL and thus participate in early atherosclerotic lesions [161]. (4) RAS is also involved in myocardial fibrosis and ventricular remodeling [162]. This activation of adipose RAS may also explain the link between excessive visceral fat and cardiovascular diseases.

Clinically, there are some drugs that inhibit RAS. ACE inhibitors such as captopril inhibit the activity of ACE and reduce the production of angiotensin II. Angiotensin II receptor antagonists such as Losartan block the angiotensin II and AT1 receptors. Renin inhibitors prevent RAS activation by suppressing the synthesis and release of renin. As RAS is related to obesity, these drugs may also prevent this disorder.

Above all, these two pathways are associated with protein folding and maturation. Protein is one of the three major nutrients needed by the body and is closely related to energy metabolism. Protein folding and maturation directly affects the conservation of energy in the body, so changes in these two pathways are of concern in the study of obesity.

5. CONCLUSION

Although many articles have studied the pathways of endothelial dysfunction associated with obesity, in this study, we have performed enrichment analysis and functional annotation for the most relevant genes through a systems biology approach. The correlation order of these pathways was obtained using a “p” value.

First, the methodology of this study identified the POMC processing in protein folding and maturation, pathway as being strongly associated with obesity. There is another pathway worthy of consideration as well: angiotensin system maturation in protein folding and maturation. So far, many pathways in obesity have been associated with cardiovascular disease; however, the two pathways above may play a greater role through various biological processes. Further clinical trials will shed light on these issues, and these pathways will likely become important targets for gene therapy and drug discovery in the future.

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References


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FIGURE 1: Enrichment analysis of Group A gene by GeneGo Meta Core: Go Pathway Maps and Go Diseases, respectively.
FIGURE 2: Enrichment analysis of Group B gene by GeneGo Meta Core: Go Pathway Maps and Go Diseases, respectively.

FIGURE 3: Protein folding and maturation POMC processing. The genes we summarize are represented by red bar histograms.
FIGURE 4: Protein folding and maturation _Angiotensin system maturation Human version. The genes we summarize in Group A gene are represented by red bar histograms.