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Analysis of vascular endothelial dysfunction genes and related pathways in obesity through systematic bioinformatics[1]

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Abstract. Obesity has become an increasingly serious health problem and popular research topic. It is associated with many diseases, especially cardiovascular disease (CVD)-related endothelial dysfunction. This study analyzed genes related to endothelial dysfunction and obesity and then summarized their most significant signaling pathways. Genes related to vascular endothelial dysfunction and obesity were extracted from a PubMed database, and analyzed by STRING, DAVID, and Gene-Go Meta-Core software. 142 genes associated with obesity were found to play a role in endothelial dysfunction in PubMed. A significant pathway (Angiotensin system maturation in protein folding and maturation) associated with obesity and endothelial dysfunction was explored. The genes and the pathway explored may play an important role in obesity. Further studies about preventing vascular endothelial dysfunction obesity should be conducted through targeting these loci and pathways.

Keywords: Pathway, obesity, vascular endothelial function, system biology

1. Introduction

The vascular endothelium is a mechanical barrier attached to blood vessels surfaces. It regulates the steady state of the cardiovascular system and is associated with almost all known cardiovascular diseases [1], such as atherosclerosis, hypertension, coronary heart disease, heart failure, etc.

Obesity is a risk factor of vascular endothelial dysfunction. Many members of the obese population have shown early symptoms of cardiovascular disease. Freedman found that systolic blood pressure and diastolic blood pressure in overweight children were 2.5 times and 3.7 times higher, respectively, than in normal weight children [2]. Glowinska found that most of the arteries in 15-year-old obese adolescents were associated with atherosclerotic lipid deposition [3]. The correlation between body fat

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distribution and cardiovascular disease has also been studied: greater deposition of central fat is associated with higher blood pressure, plasma lipid, and left ventricular mass [4].

As obesity rates rise, cardiovascular disease rates also rise. Because obesity is associated with vessel endothelial dysfunction, it is possible that preventing and treating obesity could reduce cardiovascular disease.

Genetic studies of obesity and vascular endothelial functions can provide clues about its etiology and pathogenesis to develop prevention and treatment strategies. However, previous studies did not use a system method to describe and analyze all relevant genes. This study extracted significant genes associated with obesity and vascular endothelial functions to explore their association, analyze their functions, and set up related informatics pathways. This study is a systematic summary of previous research. Further clinical verification will be reported in the next study.

2. Methods and materials

2.1. Extracting genes with statistical significance in PubMed

We searched articles in the PubMed database up to August 2014 and reviewed cited references to identify the relevant genes. Citations were retrieved "title/abstract" (keywords). Genes relating to obesity and vascular endothelial function were extracted by searching keywords as follows: "polymorphism", "gene", "genetic", "allele", "genotype"," genetics", "genome" combined with "endothelial function", "endothelium" and "obesity", "adiposis", "adipositas", "adiposity", and "fat". Gene inclusion criteria were those which could be searched in the Gene database of PubMed and effect on both of obesity and vascular endothelial function. Regardless of whether these genes extracted is in homo sapiens when they were extracted, but the genes which we researched must be in Homo sapiens.

2.2. Data analysis

This study used three methods following data analysis. STRING (http://string-db. org/) was used to analyze the connection between statistically significant genes. DAVID (http://david.abcc.ncifcrf.gov/, version: 6.7) was used to annotate the function of the candidate genes, including biological processes, function categories, and diseases. Gene-Go Meta-Core (https://portal.genego.com) was used to establish related informatics pathways of these significant genes.

3. Results

3.1. Extracting genes with statistical significance

Table 1 presents the 142 genes' characteristics.

Table 1

142 genes from PubMed database

Gene Official	Location	References	Gene Official	Location	References	Gene Official	Location	References

Symbol			Symbol			Symbol		
ABCA1	9q31.1	[5]	ACE	17q23.3	[6]	ACE2	Xp22	[7]
ADCY5	3q21.1	[8]	ADIPOQ	3q27	[9]	ADIPOR1	1q32.1	[10]
ADIPOR2	12p13.31	[10]	ADM	11p15.4	[5]	ADRB3	8p12	[10]
AGT	1q42.2	[10]	AGTR1	3q24	[6]	AGTR2	Xq22-q23	[6]
ANGPTL2	9q34	[11]	APOA5	11q23	[5]	APOC3	11q23.3	[5]
APOE	19q13.2	[5]	AR	Xq12	[10]	ARNTL	11p15	[5]
ARRDC3	5q14.3	[5]	BDNF	11p13	[8]	CAMK1D	10p13	[5]
CASR	3q13	[5]	CCL2	17q11.2-q12	[9]	CCNL1	3q25.31	[8]
CDK5	7q36	[5]	CDKAL1	6p22.3	[5]	CDKN2A	9p21	[5]
CDKN2B	9p21	[5]	CEBPA	19q13.1	[12]	CETP	16q21	[5, 10]
CFD	19p13.3	[10]	CLOCK	4q12	[5]	CRP	1q23.2	[10]
CPT1A	11q13.2	[13]	CSK	15q24.1	[5]	CYBA	16q24	[14, 15]
CYBB	Xp21.1	[16]	CYP11B2	8q21-q22	[6]	CYP17A1	10q24.3	[5]
DGAT2	11q13.5	[13]	EDN1	6p24.1	[17]	EDN2	1p34	[17]
EDN3	20q13.2-q13.3	[5, 17]	ENPP1	6q22-q23	[5, 10]	ETV5	3q28	[8]
FABP4	8q21	[10]	FAIM2	12q13	[8]	FASN	17q25	[10]
FTO	16q12.2	[8]	G6PC2	2q24.3	[5]	GCK	7p15.3-p15.1	[5]
GCKR	2p23	[5]	GH	17q24.2	[18]	GIPR	19q13.3	[5]
GNAS	20q13.3	[5]	GNPDA2	4p12	[8]	GPR98	5q13	[5]
GUCY1A3	4q31.1-q31.2	[5]	GUCY1B3	chromosome: 4	[5]	HHEX	10q23.33	[5]
HMOX1	22q13.1	[19]	HSD11B1	1q32-q41	[5, 20]	ICAM1	19p13.3-p13.2	[21]
IDE	10q23-q25	[5]	IGF2BP2	3q27.2	[5]	IL3RA	Xp22.3 or Yp11.3	[5]
IL6	7p21	[9, 10]	IL8	4q13-q21	[10]	IRS1	2q36	[5]
JAZF1	7p15.2-p15.1	[5]	KCNJ11	11p15.1	[8]	KCNQ1	11p15.5	[5]
KCTD15	19q13.11	[8]	KLF14	7q32.3	[5]	LCAT	16q22.1	[5]
LEP	7q31.3	[10]	LEPR	1p31	[10]	LIPG	18q21.1	[5]
LPL	8p22	[10]	MAS1	6q25.3-q26	[7]	MC3R	20q13.2-q13.3	[10]
MC4R	18q22	[8, 10]	MIR181B1	1q32.1	[22]	MSRA	8p23.1	[8]
MTCH2	11p11.2	[8]	MTHFR	1p36.3	[14]	MTNR1B	11q21-q22	[5]
MTTP	MTTP	[5]	NEGR1	1p31.1	[8]	NFE2L2	2q31	[23]
NFKB1	4q24	[22]	NOS3	7q36	[14]	NPPA	1p36.21	[5]
NPPB	1p36.2	[5]	NPR3	5p13.3	[5]	NR1H4	12q23.1	[5]
NR3C1	5q31.3	[10]	NR3C2	4q31.1	[5]	PARL	3q27.1	[10]
PCSK1	5q15-q21	[10]	PER2	2q37.3	[5]	PFKP	10p15.3-p15.2	[8]
PNPLA3	22q13.31	[5]	POMC	2p23.3	[10]	PPARA	22q13.31	[24]
PPARG	3p25	[13]	PPARGC1A	4p15.1	[5]	PRKAA1	5p12	[5, 25]

PTER	10p12	[8]	PTGS2	1q25.2-q25.3	[26]	RBP4	10q23-q24	[10]
RETN	19p13.2	[10]	RXRA	9q34.3	[5]	SCD	10q24.31	[10]
SCNN1A	12p13	[5]	SCNN1B	16p12.2-p12.1	[5]	SDCCAG8	1q43	[8]
SEC16B	1q25.2	[8]	SERPINE1	7q22.1	[10]	SH2B1	16p11.2	[8]
SH2B3	12q24	[5]	SHBG	17p13-p12	[27]	SHC1	1q21	[28]
SIRT1	10q21.3	[29]	SLC2A4	17p13	[10]	SLC30A8	8q24.11	[5]
SLC7A10	19q13.1	[5]	SOD1	21q22.11	[30]	SOD2	6q25.3	[30]
SOD3	4p15.2	[31]	SREBF1	17p11.2	[10, 13]	TBX3	12q24.21	[5]
TBX5	12q24.1	[5]	TCF7L2	10q25.3	[5]	TFAP2B	6p12	[8]
TMEM18	2p25.3	[8]	TNF	6p21.3	[13, 32]	TSPAN8	12q14.1-q21.1	[5]
TXN	9q31	[33]	ULK3	15q24.1	[5]	WFS1	4p16.1	[5]
WNK1	12p13.3	[5]						

3.2. STRING analysis

STRING network mapping was completed to clarify links between genes. STRING is a database of known and predicted protein interactions. These genes code the corresponding proteins, so STRING can find their interactions. As shown in Figure 1, colored links represent genes link.

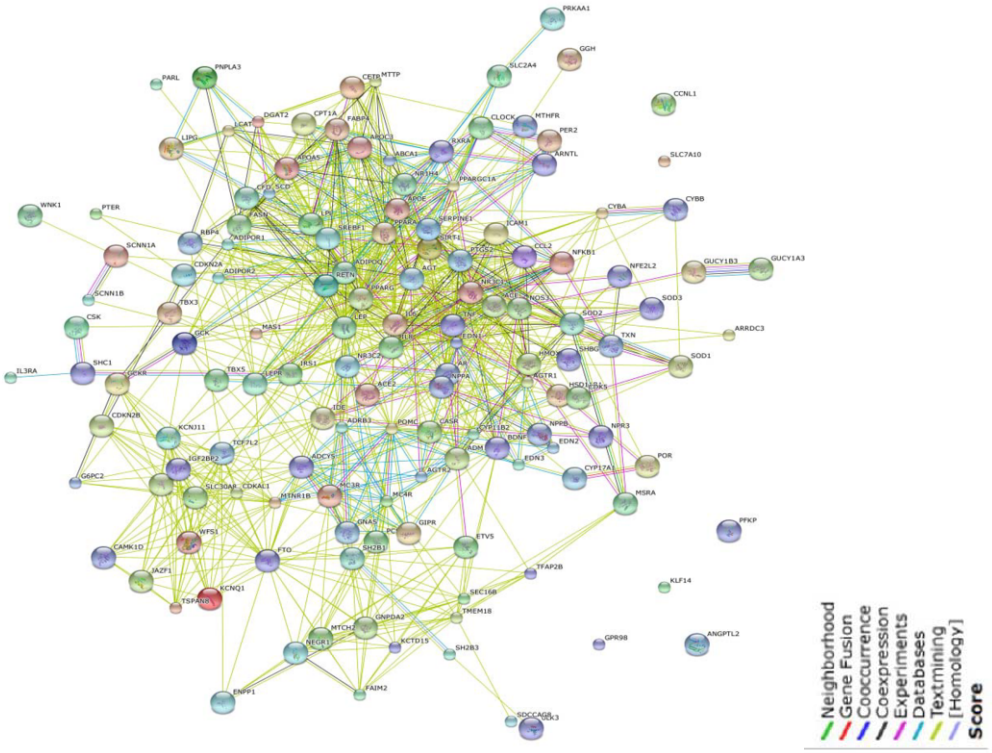

Fig. 1. STRING analysis of the relationship between genes. Different line colors represent the type of evidence for the association.

3.3. Gene functional annotation analysis

Genes with statistical significances were submitted to functional annotation analysis using DAVID software. The analyses of biological processes, functional categories, diseases and pathways were based on p value, FDR and Enrichment Score.

From the results of DAVID software, significant biological processes of these genes include response to organic substance, hormone stimulus, peptide hormone stimulus and insulin stimulus (Enrichment Score=13.07); glucose homeostasis and carbohydrate homeostasis (Enrichment Score=12.07); response to nutrient levels, extracellular stimulus and nutrient (Enrichment Score=11.08). Their functional categories significantly are in diabetes mellitus (P Value: 3.08E-18), lipid metabolism (P Value: 6.77E-08), steroid metabolism (P Value: 1.64E-05), cholesterol metabolism (P Value: 0.00124), vasoconstrictor (P Value: 2.85E-06), etc. They also participated in type 2 diabetes. Besides, related signaling pathways were renin-angiotensin system (hsa04614, P Value: 6.13E-06) and type II diabetes mellitus (hsa04930, P Value: 0.001024).

These genes were associated with hormone regulation, metabolism, and vasoconstriction. They played a role in obesity and endothelial dysfunction.

3.4. 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 showed as a higher rating for the entity. All maps were drawn with GeneGo.

As is shown in Figure 2, the most significant GeneGo pathway maps in 142 genes were (1) protein folding and maturation of POMC processing, (2) putative pathways for stimulation of

Fig. 2. Enrichment analysis of 142 genes by GeneGo meta core: go pathway maps, go diseases and go process, respectively.

fat-cell differentiation by bisphenol A, and (3) protein folding and maturation_angiotensin system maturation/human or rodent version. Other pathways were also identified, including development_Insulin, IGF-1 and TNF-alpha in brown adipocyte differentiation, development_Beta adrenergic receptors in brown adipocyte differentiation, adiponectin in pathogenesis of type 2 diabetes, regulation of metabolism_role of Adiponectin in regulation of metabolism, and so on. The most significant diseases included hyperinsulinism, insulin resistance, diabetes mellitus, type 2, etc. The most significant processes were responses to various material metabolism, blood circulation, and circulatory system processes.

Above all, most of these genes played a role in metabolism and circulation.

4. Discussion

4.1. Obesity and cardiovascular disease

Research shows that rising obesity rates pose a serious threat to public health. Obese people often show early signs of cardiovascular disease, such as early atherosclerotic vascular pathological changes, hypertension, and increased left ventricular cardiac dysfunction. As a result, there are increased mortality rates for cardiovascular disease.

4.2. The pathway of protein folding and maturation—angiotensin system maturation and cardiovascular disease

AGT, ACE, ACE2, AGTR1, and AGTR2 occupied the central position in STRING network map, which acted as main components of the angiotensin system. In the results of DAVID software, angiotensin system (P Value: 6.13E-06) was a much related pathway. In GeneGo Meta Core, the pathway of protein folding and maturation_angiotensin system maturation (P Value: 1.913E-13) was significantly enriched.

Based on these results, the pathway of protein folding and maturation_angiotensin system maturation causes great concern, and strongly correlates with vessel endothelium dysfunction in obesity.

Figure 3 shows the pathway map 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. The effects of angiotensin II and III are the strongest among these products, although the concentration of angiotensin III is lower. Therefore, angiotensin II plays a primary role in the renin-angiotensin system (RAS). Angiotensin II also can result in the formation of angiotensin (1-9) by angiotensin I converting enzyme 2 (ACE2). Then angiotensin (1-9) hydrolyze to angiotensin (1-7), which is active angiotensin peptide. Angiotensin II receptor types 1 and 2 (AGTR1 and AGTR2) and Mas1 as receptors of angiotensin (1-7) also play a role.

Unlike AGTR1 receptor which influences vasoconstriction, oxidative stress, sympathetic activation, cardiac hypertrophy, and sodium and water retention, AGTR2 and Mas1 receptors participate in vasodilation, apoptosis, and cell growth inhibition. These two opposite effects in the same pathway map relate to cardiovascular disease.

Recent studies show that the ACE-AngII-AGTR1 receptor axis and ACE2-Angiotensin-(1-7) [Ang (1-7)]-Mas receptor axis play different roles in RAS.

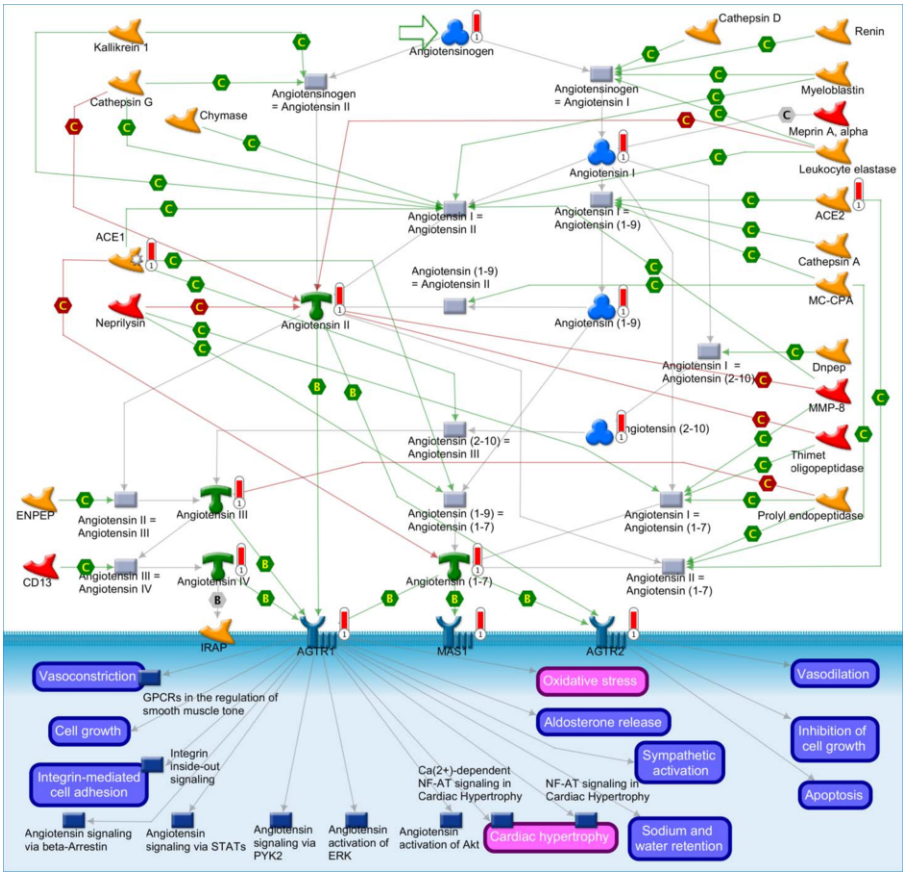

Fig. 3. The pathway map of Protein folding and maturation—angiotensin system maturation.

It is a well-demonstrated that the ACE-AngII-AGTR1 receptor axis closely correlates to cardiovascular disease: (1) For hypertension, AngII can activate endothelial NADH/NADPH oxidase and induce super oxygen anion production. AngII can also shrink blood vessels and reduce the vasodilating properties of NO. AngII and prostaglandin can stimulate endothelin release, causing vascular smooth muscle contraction, endothelial damage, smooth-muscle-cell proliferation, and vascular wall remodeling [34, 35]. (2) AGT, ACE, and AngII are significantly elevated in the myocardium of patients with chronic congestive heart failure [36]. (3) For atherosclerosis, monocytes and macrophages aggregate on vessel walls, following binding between AngII and the AGTR1 receptor, to form terminal foam cells by absorbing oxLDL and thus participate in early atherosclerotic lesions [37]. (4) RAS is also involved in myocardial fibrosis and ventricular remodeling [38]. Therefore, ACEI or ARB prevents cardiovascular events.

A new pathway consisting of the ACE2-Ang (1-7)-Mas receptor axis was discovered in 2000, which could stimulate vasodilation, anti-hypertrophy, and anti-hyperplasia. Our analysis results not only coincide with the above finding, but also show that the AGTR2 had the same effect as Mas as one of Ang (1-7) receptors.

ACE2 mRNA was significantly increased [39] in idiopathic dilated cardiomyopathy, ischemic cardiomyopathy, and heart failure patients. ACE2 may be a relevant target for treating heart failure. In all hypertensive rat strains, ACE2 mRNA and protein expression were markedly reduced [40]. In

ACE2 knockout mice, Ang II-mediated vascular remodeling was exaggerated through increased reactive oxygen species and vascular smooth muscle cell apoptosis [41]. These results for ACE2 show that it is an essential regulator of cardiovascular disease in vivo.

Cyclic Ang (1-7) lowered left ventricular end-diastolic pressure and improved endothelial functions in rats with myocardial infarction [42]. In male Lewis rats infused with Ang II or Ang (1-7), Ang (1-7) increased DUSP1 to reduce MAP kinase/Smad/fibrotic factor connective tissue growth factor (CTGF) signaling and decrease fibrosis in resistance arterioles, thereby preventing end-organ damage in chronic hypertension [43]. The results showed that Ang (1-7) had the effect of anti-fibrosis. In addition, Ang (1-7) reduced cardiomyocytes growth by activating the Mas receptor in rats [44]. Consequently, Ang (1-7) can inhibit cell growth.

The encoded protein of the Mas gene may play a role in multiple processes including hypotension, smooth muscle relaxation, and cardioprotection by mediating the effects of Ang (1-7). Recent studies have found that AVE 0991, CGEN-856, and CGEN-857 are novel peptides and are developed to stimulate the Mas receptor, preserve left ventricular contractility, prevent blood pressure elevation, and decrease urinary protein excretion [45, 46] .

Upregulating AGTR2, the other Ang (1-7) receptor, can reduce atherogenesis, possibly by modulating oxidative stress and the pro-inflammatory cascade [47]. Thus, AGTR2 may be an important therapeutic approach in atherosclerosis.

From the above, the ACE2-Ang (1-7)-Mas receptor axis and ACE-Ang II-AT1 receptor axis in RAS keeps the dynamic balance in vivo, preventing the development of cardiovascular disease.

4.3. RAS and obesity

The adipose RAS is overactivated in obesity [48], and the inhibitor of RAS attenuated obesity-induced insulin resistance in rodents. Overall, increased Ang II levels result in reduced lipolysis and increased lipogenesis, promoting triglyceride storage in adipocytes [49]. In the liver, the ACE2-Ang (1-7)-Mas axis can ameliorate insulin resistance. Activation of the ACE2-Ang(1-7)-Mas axis increases glucose uptake and decreases glycogen synthesis in the liver accompanied by increased expression of glucose transporters, insulin receptor substrates, and decreased expression of enzymes for glycogen synthesis [50] .

5. Conclusion

In this study, enrichment analysis and functional annotation have been performed for the most relevant genes and their code protein network maps through a systematic biology approach. 142 relevant genes were extracted and their significant genetic pathways were enriched. The pathway of angiotensin system is strongly associated with obesity and cardiovascular disease; this pathway may play a greater role in preventing metabolic and cardiovascular diseases. Few studies have been conducted on the ACE2-Ang (1-7)-Mas axis, which is a newly discovered pathway; currently, no drugs target this axis. Further studies should be conducted on this pathway and its potential for gene therapy.

Acknowledgments

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