Integrated DNA Methylation and Gene Expression Analysis Identified S100A8 and S100A9 in the Pathogenesis of Obesity

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Abstract

Background: To explore the association of DNA methylation and gene expression in the pathology of obesity.

Methods: (1) Genomic DNA methylation and mRNA expression profile of visceral adipose tissue (VAT) were performed in a comprehensive database of gene expression in obese and normal subjects; (2) functional enrichment analysis and construction of differential methylation gene regulatory network were performed; (3) Validation of the two different methylation sites and corresponding gene expression was done in a separate microarray data set; and (4) correlation analysis was performed on DNA methylation and mRNA expression data.

Results: A total of 77 differentially expressed mRNA matched with differentially methylated genes. Analysis revealed two different methylation sites corresponding to two unique genes-s100a8-cg09174555 and s100a9-cg03165378. Through the verification test of two interested different expression positions (DMPS) and their corresponding gene expression, we found that the methylation in these genes was negatively correlated to gene expression in the obesity group. Higher S100A8 and S100A9 expression in obese subjects were validated in a separate microarray data set.

Conclusion: This study confirmed the relationship between DNA methylation and gene expression and emphasized the important role of S100A8 and S100A9 in the pathogenesis of obesity.

Introduction

With the continuous improvement of living conditions, many countries have to pay more attention to the prevalence of obesity, because obesity has reached the proportion of epidemic that is still rising (Jensen et al. 2014). According to the World Health Organization's report in 2015, about one third or more adults are overweight, 43% of which is male and 45% as female (Collaborators et al. 2017). Obesity is implicated in many diseases, such as metabolic syndrome (MetS), type 2 diabetes (T2DM), hypertension, arteriosclerosis, cardiovascular disease (CVD), and so on (Global Burden of Metabolic Risk Factors for Chronic Diseases et al. 2014), and thus incur heavy economic burdens on countries around the world (Lozano et al. 2012). Obesity results from interactions between genetic and environmental factors, but for individuals, epigenetic factors can increase susceptibility to obesity (Sayols-Baixeras et al. 2017).

In recent years, with the deepening of research, epigenetics has emerged as a bridge between genes and environmental factors. It can change gene expression and induce long-term changes in phenotype and disease susceptibility (Smith et al. 2013). Extensive epigenomic association studies (EWAS) on DNA methylation conducted in many populations have revealed the relationship between DNA methylation and obesity (Xu et al. 2013). Changes in gene methylation changes can alter the transcription of genes resulting in abnormal gene expression and eventually obesity (Rodriguez-Rodero et al. 2017).

In the present study, we explored innovative methylation sites of obesity related DNA, by conducting an integration study using two microarray datasets, and established the relationship between the expression of obesity related genes and methylation (DEMGs). We then validated the DEMGs in a separate microarray dataset to explore the potential relationship between DNA methylation and mRNA expression on the regulation of obesity.

Materials And Methods

Gene expression profile and probe labeling

Three microarray datasets (GSE88837, GSE88940 and GSE109597) were downloaded from the gene expression database (<https://www.ncbi.nlm.nih.gov/geo/>) for analysis. GSE88837 was extracted from the U133 + 2.0 sequence of

gpl570 Affymetrix human genome for gene expression. In our study, subjects with BMI ≥ 30 were defined as obese (Jensen et al. 2014). A total of 30 subjects (including 15 obese and 15 healthy controls) were analyzed. We used the Affy package in R (Gautier et al. 2004) to convert the cel files into an expression value matrix and the RMA method to normalize the matrix. The Bioconductor package in R software was used to convert probe data into genes (Gentleman et al. 2004). If a gene corresponded to several probes, we choose the average expression value for further analysis. GSE88940 extracted from gpl13534, a human methylation 450 gene chip, was used for DNA methylation analysis, which consisted of 15 objects and 15 lean controls. All data processing was done in GEO2R (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>). In these two data sets (GSE88837 and GSE88940), there are 20 samples of the same person. We only analyze these 20 samples. GSE109597 was used as the validation data set, and the analysis method was the same as used for GSE 88837.

Differential expression and analysis of methylated genes (DEMGs)

We compared obese with control subjects to explore the differential expression genes (DEGs) of the marginal envelope in R (Miao et al. 2019). The threshold value was set as $|\log_2 \text{fold change}| \geq 2$, $P < 0.05$. GEO2R was used to determine the methylation sites (DMPS) by comparing the differences between normal and obese subjects. DMPS located in gene regions were assigned to corresponding genes, which were defined as differentially methylated genes (DMGs). The threshold value was set as $|\log_2 \text{fold-change}| (\Delta\beta) > 0.05$, $P < 0.05$. Then, we matched the DEGs with DMGs, and only the matched genes (DEMGs) were selected for further analysis.

Functional enrichment analysis

All functional enrichment analysis on DEGs was performed on cluster profiler and dose package in R (Yu et al. 2012). The complete functional enrichment analysis includes gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) approach, and Disease Ontology (DO). The threshold value of analysis was set as $\text{adjust-p} < 0.05$ and error detection rate ($\text{FDR} < 0.05$).

Protein-protein interaction (PPI) network and module analysis

We used the string database (version 11.0) (Szklarczyk et al. 2019) to explore protein prediction and experimental interactions. There are many methods of database prediction, including co-expression experiment, text mining, co-occurrence, gene fusion, database, and neighborhood. , we used the combination fraction to reveal the protein pair interactions in the database. Then, we localized DEMGs to PPIs to identify the key genes in the network with the cut-off value set to a comprehensive score > 0.9 (Miao et al. 2018). As a valuable method, a degree is used to study the role of protein nodes in the network. Using the molecular complex detection (MCODE) on Cytoscape (version 3.71), the most significant clustering module and the main clustering module were explored (Shannon et al. 2003; Bader et al. 2003). For further analysis, we set $\text{ease} \leq 0.05$ and $\text{set} \geq 2$ as the cutoff value and MCODE score > 8 as the threshold value.

Validation of DEMGs

We used prism 8.0 GraphPad (Miao et al. 2019) for scatterplots of methylation and gene expression to detect the relationship between methylation and gene expression. Then calculate the correlation equation to judge whether the equation has statistical significance. The DEMGs were validated in GSE109597, which contained 84 unrelated samples. After grouping according to BMI (> 30 and < 30), the expression of DMEGs in the two groups was compared with ggplot2 in R.

Result

Data preprocessing

A quality control processing of GSE88837 showed that all samples were normal (Fig. 1). We obtained 54560 expression probes from each gene map and expression. Probes with too low or too high probe expression were defined as outliers and eliminated from further analysis. An average expression value was used to screen the differentially expressed genes to prevent too many probes corresponding to one gene. The limma software package was used to calculate the DEGs, which yielded 1814 DEGs with P values < 0.05. Among these, 150 with a log2 (fold change) > 2 were defined as up-regulated, and 199 with a log2 (fold change) < 2 as down-regulated. The heat map and volcano map of DEGs are shown in Fig. 2 (A and B).

Out of the 485579 DNA methylation sites in GSE88940 visceral adipose tissue (VAT) that were screened for quality control, 454325 methylation sites were selected for analysis. 10016 DMPS ($|\Delta\beta| > 0.05$, $P < 0.05$) were identified, of which 666 were hypermethylated and 3349 were hypomethylated. After annotation, 10016 DMPS are located in 4024 unique genes, which were identified as differentially methylated genes (DMGs). The thermal and volcanic maps of the DMGs are shown in Fig. 2 (C and D).

Matching DMGs with DEGs yielded 77 genes for the next analysis (Fig. 3). The details of 77 genes are shown in Table 1 and Fig. 4.

Table 1
The matched pairs of DEG and DMP (Table 1. Should appear in line 167 of the manuscript)

DMPs						DEGs			
SYMBOL	CpG site	START	END	CHR	Position	$\Delta\beta$	<i>P</i> values	FC	<i>P</i> values
ADH4	cg12011299	100065546	100065669		TSS200	7.95E-02	1.13E-02	-4.15	4.70E-03
ASPHD1	cg05192831	29913007	29913130	16	1stExon	5.42E-02	1.13E-02	-5.50	2.21E-02
ASPHD1	cg02488299	29913223	29913346	16	1stExon	6.23E-02	1.13E-02	-5.50	2.21E-02
BNC1	cg27169020	83954229	83954352	15	TSS1500	7.85E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg23741520	83954231	83954354	15	TSS1500	7.86E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg12250049	83951663	83951786	15	Body	8.56E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg10275315	83954246	83954369	15	TSS1500	9.20E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg00768409	83954392	83954515	15	TSS1500	9.23E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg16049391	83954395	83954518	15	TSS1500	1.06E-01	1.13E-02	-2.54	3.92E-02
C1orf87	cg17238766	60539675	60539798	1	TSS1500	8.87E-02	1.13E-02	3.84	1.19E-02
C1orf87	cg09851465	60539671	60539794	1	TSS1500	1.07E-01	1.13E-02	3.84	1.19E-02
C3orf22	cg07743179	126278943	126279066	3	TSS1500	-5.14E-02	1.13E-02	2.63	4.95E-02
CFB	cg12124018	31916683	31916806	6	Body	-6.73E-02	1.13E-02	-3.60	1.23E-04
CFB	cg21606287	31916705	31916828	6	Body	-6.17E-02	1.13E-02	-3.60	1.23E-04
CGN	cg07596668	151509825	151509948	1	3'UTR	8.20E-02	1.13E-02	-4.83	1.38E-02
CGN	cg25198049	151509723	151509846	1	Body	8.29E-02	1.13E-02	-4.83	1.38E-02

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

Supplementary Table 1. The analysis for Gene Ontology

Supplementary Table 2. The analysis for KEGG pathway

Supplementary Table 3. The analysis for Disease Ontology

DMPs						DEGs			
CHI3L1	cg14085262	203155938	203156061	1	TSS200	5.79E-02	1.13E-02	-3.09	1.38E-02
CRB2	cg15922174	126135998	126136121	9	Body	-8.61E-02	1.13E-02	-4.69	3.40E-03
CRB2	cg02625222	126135169	126135292	9	Body	5.30E-02	1.13E-02	-4.69	3.40E-03
CRB2	cg11431402	126135408	126135531	9	Body	5.76E-02	1.13E-02	-4.69	3.40E-03
CRB2	cg13884995	126126377	126126500	9	Body	7.19E-02	1.13E-02	-4.69	3.40E-03
DMRTA1	cg14338345	22447679	22447802	9	1stExon	5.36E-02	1.13E-02	4.92	1.79E-02
DUSP1	cg08452061	172199642	172199765	5	TSS1500	5.88E-02	1.13E-02	5.41	6.80E-03
EPHA1	cg21294616	143093848	143093971	7	Body	-6.88E-02	1.13E-02	3.61	3.53E-02
EPHA1	cg26960083	143106298	143106421	7	TSS1500	6.90E-02	1.13E-02	3.61	3.53E-02
ESPN	cg13284574	6519923	6520046	1	Body	7.33E-02	1.13E-02	4.19	2.59E-02
FAM84A	cg12050497	14773274	14773397	2	5'UTR	9.44E-02	1.13E-02	-4.84	9.04E-03
FGF9	cg03688324	22251017	22251140	13	Body	6.28E-02	1.13E-02	-2.68	1.25E-02
FNDC1	cg06764804	159654028	159654151	6	Body	5.46E-02	1.13E-02	-3.50	1.97E-02
GPR143	cg19318920	9693690	9693813	X	3'UTR	5.73E-02	1.13E-02	-2.33	3.60E-02
GPRIN3	cg02734358	90227074	90227197	4	5'UTR	1.06E-01	1.13E-02	-4.03	2.18E-02
GSC	cg01695643	95237330	95237453	14	TSS1500	8.16E-02	1.13E-02	2.62	3.78E-02
GSC	cg15440688	95237637	95237760	14	TSS1500	9.33E-02	1.13E-02	2.62	3.78E-02
ITGA2B	cg14686645	42452426	42452549	17	Body	6.75E-02	1.13E-02	2.59	1.34E-02

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

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DMPs						DEGs			
KCNK3	cg19115882	26919145	26919268	2	Body	5.71E-02	2.04E-03	-2.31	4.83E-03
KCNK3	cg19991086	26953767	26953890	2	3'UTR	6.04E-02	2.04E-03	-2.31	4.83E-03
KCNN3	cg16296829	154832535	154832658	2	TSS200	6.21E-02	2.04E-03	-2.31	4.83E-03
KCNN3	cg18315680	154833117	154833240	2	Body	8.39E-02	2.04E-03	-2.31	4.83E-03
KLF2	cg02668248	16437789	16437912	19	Body	-7.35E-02	2.04E-03	3.95	1.90E-02
KLHL34	cg20312916	21676605	21676728	X	TSS200	-6.58E-02	2.04E-03	3.99	1.49E-02
KLHL34	cg01828474	21676593	21676716	X	TSS200	-5.27E-02	2.04E-03	3.99	1.49E-02
KLHL34	cg25075572	21673930	21674053	X	1stExon	-5.05E-02	2.04E-03	3.99	1.49E-02
KRT7	cg25313172	52627272	52627395	12	1stExon	-6.67E-02	2.04E-03	-3.94	2.60E-02
KRT7	cg14537533	52626904	52627027	12	TSS200	-6.14E-02	2.04E-03	-3.94	2.60E-02
KRT7	cg07967679	52626814	52626937	12	TSS200	-5.58E-02	2.04E-03	-3.94	2.60E-02
KRT7	cg07022048	52638592	52638715	12	Body	6.70E-02	2.04E-03	-3.94	2.60E-02
KRT71	cg23767977	52947465	52947588	15	TSS1500	5.19E-02	2.04E-03	3.47	3.17E-02
KRT8	cg24504361	53297987	53298110	12	Body	6.83E-02	2.04E-03	-2.20	4.88E-03
LAD1	cg11418783	201369650	201369773	1	TSS1500	5.47E-02	2.04E-03	2.97	1.95E-02
MARCO	cg07554474	119698443	119698566	2	TSS1500	6.99E-02	2.04E-03	-2.45	1.73E-02
MC2R	cg25924472	13884152	13884275	18	3'UTR	-1.15E-01	2.04E-03	4.96	3.62E-03
MFAP4	cg15119221	19290755	19290878	17	TSS1500	6.26E-02	2.04E-03	2.22	3.19E-02

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

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DMPs						DEGs			
MYT1L	cg14128411	1926907	1927030	2	Body	-1.08E-01	2.04E-03	-2.32	4.16E-02
MYT1L	cg00067742	1926888	1927011	2	Body	-7.81E-02	2.04E-03	-2.32	4.16E-02
MYT1L	cg09022325	1796228	1796351	2	Body	5.95E-02	2.04E-03	-2.32	4.16E-02
MYT1L	cg22388316	2000192	2000315	2	5'UTR	8.38E-02	2.04E-03	-2.32	4.16E-02
NGFR	cg04466214	47581280	47581403	17	Body	5.49E-02	2.04E-03	-2.34	4.35E-03
NGFR	cg17369032	47590326	47590449	17	Body	7.93E-02	2.04E-03	-2.34	4.35E-03
PCDHB3	cg01925738	140480770	140480893	5	1stExon	-7.09E-02	2.04E-03	3.59	1.00E-02
PKD2L2	cg10535132	137224284	137224407	5	TSS1500	6.68E-02	2.04E-03	-3.16	2.52E-02
ROR2	cg14244439	94561075	94561198	9	Body	5.75E-02	2.04E-03	-2.71	2.79E-02
ROR2	cg02785332	94647670	94647793	9	Body	6.55E-02	2.04E-03	-2.71	2.79E-02
RXFP1	cg03875996	159442782	159442905	4	TSS1500	7.56E-02	2.04E-03	4.77	6.47E-03
S100A8	cg09174555	153364020	153364143	1	TSS1500	7.03E-02	2.04E-03	-2.25	8.00E-03
S100A9	cg03165378	153329882	153330005	1	TSS1500	7.51E-02	2.04E-03	-2.05	1.13E-02
SCTR	cg26009192	120282200	120282323	2	TSS200	-5.88E-02	2.04E-03	2.45	4.12E-02
SGPP2	cg14435109	223288714	223288837	2	TSS1500	5.72E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg07873848	223288635	223288758	2	TSS1500	6.25E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg11300809	223288637	223288760	2	TSS1500	7.03E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg10091265	223288331	223288454	2	TSS1500	7.16E-02	2.04E-03	-3.25	2.72E-03

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

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DMPs						DEGs			
SGPP2	cg16171484	223290515	223290638	2	Body	7.99E-02	2.04E-03	-3.25	2.72E-03
SMPD3	cg07735969	68418473	68418596	16	5'UTR	6.49E-02	2.04E-03	-3.52	2.63E-02
SRGN	cg18278184	70847430	70847553	10	TSS1500	8.69E-02	2.04E-03	3.49	4.83E-02
TNRC18	cg06947694	5389271	5389394	7	Body	-7.03E-02	2.04E-03	-2.58	1.12E-02
TNRC18	cg19679210	5354874	5354997	7	Body	6.58E-02	2.04E-03	-2.58	1.12E-02
TNXB	cg02657865	32077744	32077867	6	TSS1500	-8.95E-02	2.04E-03	2.82	2.92E-02
TNXB	cg13199127	32049196	32049319	6	Body	-8.53E-02	2.04E-03	2.82	2.92E-02
TNXB	cg25596754	32026610	32026733	6	Body	-7.77E-02	2.04E-03	2.82	2.92E-02
TNXB	cg00661399	32049177	32049300	6	Body	-7.60E-02	2.04E-03	2.82	2.92E-02
TNXB	cg24336152	32070785	32070908	6	5'UTR	-7.03E-02	2.04E-03	2.82	2.92E-02
TNXB	cg14669361	32038747	32038870	6	Body	-6.91E-02	0.00589	2.82	2.92E-02
TNXB	cg20858622	32015651	32015774	6	Body	-6.80E-02	9.84E-03	2.82	2.92E-02
TNXB	cg11493661	32016239	32016362	6	Body	-6.70E-02	5.63E-03	2.82	2.92E-02
TNXB	cg05956076	32074934	32075057	6	5'UTR	-6.55E-02	2.97E-02	2.82	2.92E-02
TNXB	cg16478197	32068181	32068304	6	5'UTR	-6.32E-02	2.04E-03	2.82	2.92E-02
TNXB	cg12493058	32052444	32052567	6	Body	5.59E-02	1.69E-02	2.82	2.92E-02
TNXB	cg20928974	32022642	32022765	6	Body	5.68E-02	1.97E-02	2.82	2.92E-02
TNXB	cg16662408	32053637	32053760	6	Body	5.95E-02	2.36E-02	2.82	2.92E-02

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

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DMPs						DEGs			
TNXB	cg23636802	32054441	32054564	6	Body	7.13E-02	2.36E-02	2.82	2.92E-02
TNXB	cg13606255	32053100	32053223	6	Body	7.94E-02	2.36E-02	2.82	2.92E-02
TNXB	cg13739666	32013974	32014097	6	TSS200	-7.87E-02	2.36E-02	2.82	2.92E-02
TNXB	cg26537323	32014059	32014182	6	TSS200	-7.60E-02	2.36E-02	2.82	2.92E-02
TNXB	cg18178844	32014100	32014223	6	TSS200	-6.24E-02	2.36E-02	2.82	2.92E-02
TNXB	cg25522795	32014096	32014219	6	TSS200	-5.09E-02	2.36E-02	2.82	2.92E-02
TNXB	cg15376677	32011687	32011810	6	Body	5.40E-02	2.36E-02	2.82	2.92E-02
TNXB	cg18340416	32010178	32010301	6	Body	5.89E-02	2.36E-02	2.82	2.92E-02
TNXB	cg20161227	31980836	31980959	6	5'UTR	-7.46E-02	2.36E-02	2.82	2.92E-02
TRIML1	cg04086012	189060900	189061023	4	1stExon	5.25E-02	2.36E-02	-3.35	2.19E-02
UNC13C	cg06530558	54304778	54304901	15	TSS1500	5.72E-02	2.36E-02	2.07	4.67E-02
VWA5B1	cg05805297	20617329	20617452	1	TSS200	7.04E-02	2.36E-02	4.25	1.29E-02
VWA5B1	cg04637372	20617456	20617579	1	5'UTR	7.54E-02	2.36E-02	4.25	1.29E-02
VWA5B1	cg25745746	20617452	20617575	1	5'UTR	8.04E-02	2.36E-02	4.25	1.29E-02
WNK4	cg06795963	40932359	40932482	17	TSS1500	-7.78E-02	2.36E-02	-3.68	1.54E-02
ZDHC1	cg06139166	67440283	67440406	16	Body	-1.02E-01	2.36E-02	2.14	1.65E-02
ZDHC1	cg08968184	67433636	67433759	16	Body	5.10E-02	2.36E-02	2.14	1.65E-02
ZFR2	cg09999510	3812479	3812602	19	Body	5.01E-02	2.36E-02	2.71	3.06E-02
CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change									
Supplementary Table 1. The analysis for Gene Ontology									
Supplementary Table 2. The analysis for KEGG pathway									
Supplementary Table 3. The analysis for Disease Ontology									

Table 1
The matched pairs of DEG and DMP

DMPs							DEGs		
SYMBOL	CpG site	START	END	CHR	Position	$\Delta\beta$	<i>P</i> values	FC	<i>P</i> values
ADH4	cg12011299	100065546	100065669		TSS200	7.95E-02	1.13E-02	-4.15	4.70E-03
ASPHD1	cg05192831	29913007	29913130	16	1stExon	5.42E-02	1.13E-02	-5.50	2.21E-02
ASPHD1	cg02488299	29913223	29913346	16	1stExon	6.23E-02	1.13E-02	-5.50	2.21E-02
BNC1	cg27169020	83954229	83954352	15	TSS1500	7.85E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg23741520	83954231	83954354	15	TSS1500	7.86E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg12250049	83951663	83951786	15	Body	8.56E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg10275315	83954246	83954369	15	TSS1500	9.20E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg00768409	83954392	83954515	15	TSS1500	9.23E-02	1.13E-02	-2.54	3.92E-02
BNC1	cg16049391	83954395	83954518	15	TSS1500	1.06E-01	1.13E-02	-2.54	3.92E-02
C1orf87	cg17238766	60539675	60539798	1	TSS1500	8.87E-02	1.13E-02	3.84	1.19E-02
C1orf87	cg09851465	60539671	60539794	1	TSS1500	1.07E-01	1.13E-02	3.84	1.19E-02
C3orf22	cg07743179	126278943	126279066	3	TSS1500	-5.14E-02	1.13E-02	2.63	4.95E-02
CFB	cg12124018	31916683	31916806	6	Body	-6.73E-02	1.13E-02	-3.60	1.23E-04
CFB	cg21606287	31916705	31916828	6	Body	-6.17E-02	1.13E-02	-3.60	1.23E-04
CGN	cg07596668	151509825	151509948	1	3'UTR	8.20E-02	1.13E-02	-4.83	1.38E-02
CGN	cg25198049	151509723	151509846	1	Body	8.29E-02	1.13E-02	-4.83	1.38E-02
CHI3L1	cg14085262	203155938	203156061	1	TSS200	5.79E-02	1.13E-02	-3.09	1.38E-02
CRB2	cg15922174	126135998	126136121	9	Body	-8.61E-02	1.13E-02	-4.69	3.40E-03

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

DMPs						DEGs			
CRB2	cg02625222	126135169	126135292	9	Body	5.30E-02	1.13E-02	-4.69	3.40E-03
CRB2	cg11431402	126135408	126135531	9	Body	5.76E-02	1.13E-02	-4.69	3.40E-03
CRB2	cg13884995	126126377	126126500	9	Body	7.19E-02	1.13E-02	-4.69	3.40E-03
DMRTA1	cg14338345	22447679	22447802	9	1stExon	5.36E-02	1.13E-02	4.92	1.79E-02
DUSP1	cg08452061	172199642	172199765	5	TSS1500	5.88E-02	1.13E-02	5.41	6.80E-03
EPHA1	cg21294616	143093848	143093971	7	Body	-6.88E-02	1.13E-02	3.61	3.53E-02
EPHA1	cg26960083	143106298	143106421	7	TSS1500	6.90E-02	1.13E-02	3.61	3.53E-02
ESPN	cg13284574	6519923	6520046	1	Body	7.33E-02	1.13E-02	4.19	2.59E-02
FAM84A	cg12050497	14773274	14773397	2	5'UTR	9.44E-02	1.13E-02	-4.84	9.04E-03
FGF9	cg03688324	22251017	22251140	13	Body	6.28E-02	1.13E-02	-2.68	1.25E-02
FNDC1	cg06764804	159654028	159654151	6	Body	5.46E-02	1.13E-02	-3.50	1.97E-02
GPR143	cg19318920	9693690	9693813	X	3'UTR	5.73E-02	1.13E-02	-2.33	3.60E-02
GPRIN3	cg02734358	90227074	90227197	4	5'UTR	1.06E-01	1.13E-02	-4.03	2.18E-02
GSC	cg01695643	95237330	95237453	14	TSS1500	8.16E-02	1.13E-02	2.62	3.78E-02
GSC	cg15440688	95237637	95237760	14	TSS1500	9.33E-02	1.13E-02	2.62	3.78E-02
ITGA2B	cg14686645	42452426	42452549	17	Body	6.75E-02	1.13E-02	2.59	1.34E-02
KCNK3	cg19115882	26919145	26919268	2	Body	5.71E-02	2.04E-03	-2.31	4.83E-03
KCNK3	cg19991086	26953767	26953890	2	3'UTR	6.04E-02	2.04E-03	-2.31	4.83E-03
KCNN3	cg16296829	154832535	154832658	2	TSS200	6.21E-02	2.04E-03	-2.31	4.83E-03
KCNN3	cg18315680	154833117	154833240	2	Body	8.39E-02	2.04E-03	-2.31	4.83E-03

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

DMPs						DEGs			
KLF2	cg02668248	16437789	16437912	19	Body	-7.35E-02	2.04E-03	3.95	1.90E-02
KLHL34	cg20312916	21676605	21676728	X	TSS200	-6.58E-02	2.04E-03	3.99	1.49E-02
KLHL34	cg01828474	21676593	21676716	X	TSS200	-5.27E-02	2.04E-03	3.99	1.49E-02
KLHL34	cg25075572	21673930	21674053	X	1stExon	-5.05E-02	2.04E-03	3.99	1.49E-02
KRT7	cg25313172	52627272	52627395	12	1stExon	-6.67E-02	2.04E-03	-3.94	2.60E-02
KRT7	cg14537533	52626904	52627027	12	TSS200	-6.14E-02	2.04E-03	-3.94	2.60E-02
KRT7	cg07967679	52626814	52626937	12	TSS200	-5.58E-02	2.04E-03	-3.94	2.60E-02
KRT7	cg07022048	52638592	52638715	12	Body	6.70E-02	2.04E-03	-3.94	2.60E-02
KRT71	cg23767977	52947465	52947588	15	TSS1500	5.19E-02	2.04E-03	3.47	3.17E-02
KRT8	cg24504361	53297987	53298110	12	Body	6.83E-02	2.04E-03	-2.20	4.88E-03
LAD1	cg11418783	201369650	201369773	1	TSS1500	5.47E-02	2.04E-03	2.97	1.95E-02
MARCO	cg07554474	119698443	119698566	2	TSS1500	6.99E-02	2.04E-03	-2.45	1.73E-02
MC2R	cg25924472	13884152	13884275	18	3'UTR	-1.15E-01	2.04E-03	4.96	3.62E-03
MFAP4	cg15119221	19290755	19290878	17	TSS1500	6.26E-02	2.04E-03	2.22	3.19E-02
MYT1L	cg14128411	1926907	1927030	2	Body	-1.08E-01	2.04E-03	-2.32	4.16E-02
MYT1L	cg00067742	1926888	1927011	2	Body	-7.81E-02	2.04E-03	-2.32	4.16E-02
MYT1L	cg09022325	1796228	1796351	2	Body	5.95E-02	2.04E-03	-2.32	4.16E-02
MYT1L	cg22388316	2000192	2000315	2	5'UTR	8.38E-02	2.04E-03	-2.32	4.16E-02
NGFR	cg04466214	47581280	47581403	17	Body	5.49E-02	2.04E-03	-2.34	4.35E-03
NGFR	cg17369032	47590326	47590449	17	Body	7.93E-02	2.04E-03	-2.34	4.35E-03

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

DMPs						DEGs			
PCDHB3	cg01925738	140480770	140480893	5	1stExon	-7.09E-02	2.04E-03	3.59	1.00E-02
PKD2L2	cg10535132	137224284	137224407	5	TSS1500	6.68E-02	2.04E-03	-3.16	2.52E-02
ROR2	cg14244439	94561075	94561198	9	Body	5.75E-02	2.04E-03	-2.71	2.79E-02
ROR2	cg02785332	94647670	94647793	9	Body	6.55E-02	2.04E-03	-2.71	2.79E-02
RXFP1	cg03875996	159442782	159442905	4	TSS1500	7.56E-02	2.04E-03	4.77	6.47E-03
S100A8	cg09174555	153364020	153364143	1	TSS1500	7.03E-02	2.04E-03	-2.25	8.00E-03
S100A9	cg03165378	153329882	153330005	1	TSS1500	7.51E-02	2.04E-03	-2.05	1.13E-02
SCTR	cg26009192	120282200	120282323	2	TSS200	-5.88E-02	2.04E-03	2.45	4.12E-02
SGPP2	cg14435109	223288714	223288837	2	TSS1500	5.72E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg07873848	223288635	223288758	2	TSS1500	6.25E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg11300809	223288637	223288760	2	TSS1500	7.03E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg10091265	223288331	223288454	2	TSS1500	7.16E-02	2.04E-03	-3.25	2.72E-03
SGPP2	cg16171484	223290515	223290638	2	Body	7.99E-02	2.04E-03	-3.25	2.72E-03
SMPD3	cg07735969	68418473	68418596	16	5'UTR	6.49E-02	2.04E-03	-3.52	2.63E-02
SRGN	cg18278184	70847430	70847553	10	TSS1500	8.69E-02	2.04E-03	3.49	4.83E-02
TNRC18	cg06947694	5389271	5389394	7	Body	-7.03E-02	2.04E-03	-2.58	1.12E-02
TNRC18	cg19679210	5354874	5354997	7	Body	6.58E-02	2.04E-03	-2.58	1.12E-02
TNXB	cg02657865	32077744	32077867	6	TSS1500	-8.95E-02	2.04E-03	2.82	2.92E-02
TNXB	cg13199127	32049196	32049319	6	Body	-8.53E-02	2.04E-03	2.82	2.92E-02
TNXB	cg25596754	32026610	32026733	6	Body	-7.77E-02	2.04E-03	2.82	2.92E-02

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

DMPs						DEGs			
TNXB	cg00661399	32049177	32049300	6	Body	-7.60E-02	2.04E-03	2.82	2.92E-02
TNXB	cg24336152	32070785	32070908	6	5'UTR	-7.03E-02	2.04E-03	2.82	2.92E-02
TNXB	cg14669361	32038747	32038870	6	Body	-6.91E-02	0.00589	2.82	2.92E-02
TNXB	cg20858622	32015651	32015774	6	Body	-6.80E-02	9.84E-03	2.82	2.92E-02
TNXB	cg11493661	32016239	32016362	6	Body	-6.70E-02	5.63E-03	2.82	2.92E-02
TNXB	cg05956076	32074934	32075057	6	5'UTR	-6.55E-02	2.97E-02	2.82	2.92E-02
TNXB	cg16478197	32068181	32068304	6	5'UTR	-6.32E-02	2.04E-03	2.82	2.92E-02
TNXB	cg12493058	32052444	32052567	6	Body	5.59E-02	1.69E-02	2.82	2.92E-02
TNXB	cg20928974	32022642	32022765	6	Body	5.68E-02	1.97E-02	2.82	2.92E-02
TNXB	cg16662408	32053637	32053760	6	Body	5.95E-02	2.36E-02	2.82	2.92E-02
TNXB	cg23636802	32054441	32054564	6	Body	7.13E-02	2.36E-02	2.82	2.92E-02
TNXB	cg13606255	32053100	32053223	6	Body	7.94E-02	2.36E-02	2.82	2.92E-02
TNXB	cg13739666	32013974	32014097	6	TSS200	-7.87E-02	2.36E-02	2.82	2.92E-02
TNXB	cg26537323	32014059	32014182	6	TSS200	-7.60E-02	2.36E-02	2.82	2.92E-02
TNXB	cg18178844	32014100	32014223	6	TSS200	-6.24E-02	2.36E-02	2.82	2.92E-02
TNXB	cg25522795	32014096	32014219	6	TSS200	-5.09E-02	2.36E-02	2.82	2.92E-02
TNXB	cg15376677	32011687	32011810	6	Body	5.40E-02	2.36E-02	2.82	2.92E-02
TNXB	cg18340416	32010178	32010301	6	Body	5.89E-02	2.36E-02	2.82	2.92E-02
TNXB	cg20161227	31980836	31980959	6	5'UTR	-7.46E-02	2.36E-02	2.82	2.92E-02
TRIML1	cg04086012	189060900	189061023	4	1stExon	5.25E-02	2.36E-02	-3.35	2.19E-02

CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change

DMPs						DEGs			
UNC13C	cg06530558	54304778	54304901	15	TSS1500	5.72E-02	2.36E-02	2.07	4.67E-02
VWA5B1	cg05805297	20617329	20617452	1	TSS200	7.04E-02	2.36E-02	4.25	1.29E-02
VWA5B1	cg04637372	20617456	20617579	1	5'UTR	7.54E-02	2.36E-02	4.25	1.29E-02
VWA5B1	cg25745746	20617452	20617575	1	5'UTR	8.04E-02	2.36E-02	4.25	1.29E-02
WNK4	cg06795963	40932359	40932482	17	TSS1500	-7.78E-02	2.36E-02	-3.68	1.54E-02
ZDHHC1	cg06139166	67440283	67440406	16	Body	-1.02E-01	2.36E-02	2.14	1.65E-02
ZDHHC1	cg08968184	67433636	67433759	16	Body	5.10E-02	2.36E-02	2.14	1.65E-02
ZFR2	cg09999510	3812479	3812602	19	Body	5.01E-02	2.36E-02	2.71	3.06E-02
CHR, chromosome; DEG, differentially expressed gene; $\Delta\beta$, difference of methylation between patients with Obesity and healthy controls; DMP, differential methylation position; START/END, position in Build 37; FC, log fold change									

Functional Annotation

The cluster profiler package in R for DO function, KEGG pathway enrichment, and GO analysis was used to clarify the role of the DEGs. 104 cell components, 7 molecular functions, and 329 biological processes were significantly enriched in GO (corrected $P < 0.05$, Fig. 5A and Supplementary Table 1). Also, 22 pathways were enriched in KEGG, and 77 DO items were identified at $p < 0.05$ after correction (error detection rate, $FDR < 0.05$). The results are shown in Fig. 5 (B and C) and Supplementary Tables 2 and 3.

Enrichments including GO: 0061448 connective tissue development, GO: 0007584 nutritional response, GO: 0022600 digestive system process, GO: 0045444 adipocyte differentiation, hsa04010 MAPK signal pathway, hsa04657 IL-17 signal pathway, hsa0061 fatty acid biosynthesis, hsa04933 age-old signal pathway in diabetic complications, DOID: 9352 Type 2 diabetes mellitus; DOID: 374 nutritional diseases; DOID: 654 over nutrition; DOID: 9970 obesity have been previously reported to be related to obesity, and thus genes in these clusters were selected for further analysis.

Construction Of Ppi Network And Identification Of Key Genes

The string database was used to clarify gene interaction networks of the selected genes on Cytoscape, which revealed 5598 pairs of proteins and 1003 nodes when the cut-off value set to a comprehensive score > 0.9 (Fig. 6A). MCODE analysis showed the scores of the four modules were more than 10 (Fig. 6B-E). Analysis of 98 genes in these four modules revealed that two of them were highly correlated, and carried out submodule analysis to screen out GO, DO, and KEGG data. The two genes were S100 calcium-binding protein A8 (S100A8) and S100 calcium-binding protein A9 (S100A9).

Hub Genes Validation

First, we tested the relationship between S100A8 and S100A9 methylation and gene expression in the same 20 individuals in GSE88837 and GSE88940. We found that there was a negative correlation between methylation level and gene expression. The results also showed that the two correlation equation lines were statistically significant ($P < 0.05$) (Fig. 7). Validation in GSE105597 showed that the expression of S100A8 and S100A9 was significantly higher in obese vs. normal subjects ($P < 0.05$, Fig. 8).

Discussion

Emerging evidence has shown that obesity is not only a simple nutritional excess but also a metabolic disorder, which is the precursor of many metabolic diseases (Force et al. 2018). More and more studies have confirmed that chronic inflammation is the result of the accumulation of fat cells caused by abnormal gene function (Yamaoka et al. 2014). In the present study, the relationship between gene expression and obesity in 20 obese patients was analyzed using a gene-chip dataset. We found that the high expression of S100A8 and S100A9 was related to obesity. Also, a significant increase in S100A8 and S100A9 expression was observed in the validation microarray dataset of 84 samples. Whole-genome methylation analysis showed hypomethylation of the S100A8 and S100A9 promoters. Changes in methylation often lead to abnormal gene function and diseases and in this case, maybe an important cause of obesity.

Calprotectin is a heterodimer composed of S100A8 (calprotectin a, MRP8) and S100A9 (calprotectin B, MRP14) subunits, which are low molecular weight members of S100 calprotectin subfamily (Catalan et al. 2011). Previous studies have found that S100A8 and S100A9 are related to obesity, insulin resistance, and atherosclerosis (Yamaoka et al. 2013). Sekimoto et al. found that serum S100A8 / A9 complex level was related to leukocyte count, BMI, subcutaneous fat area, and visceral fat area. At the same time, compared with lean mice, obese mice had higher S100A8 mRNA expression in mature adipocyte component and obese mice had higher S100A9 expression in matrix vascular component (Sekimoto et al. 2012). Louise Lyloff et al. found that Roux-en-Y gastric bypass surgery (RYGB) significantly decreased BMI, and circulating mRNA levels of S100A8 and S100A9 in obese patients (Lylloff et al. 2017). These findings are consistent with the present findings.

Epigenetics has become an intense topic of research in recent years. It refers to the regulation of gene expression without changing the basic structure of the gene. At present, epigenetics usually refers to histone modification, and methylation of noncoding RNA, and DNA (Wahl et al. 2017; Dick et al. 2014). Many studies have found that epigenetic mechanisms are associated with obesity. Sonne et al. found changes in methylation and expression of nine genes in epididymal adipocytes, including *ehd2* and *kctd15*, which are known to be obesity-related genes, and a new candidate gene *IRF8*, which may be related to the 1 / 2 balance of immune type. Similarly, Dijk and Bell et al. also explained the relationship between DNA methylation and obesity. It is worth noting that these two studies are comprehensive in content, more stringent in inclusion criteria, extensive in research content, and very reliable in conclusions (Bell et al. 2017; van Dijk et al. 2015). Benton et al. demonstrated the relationship between DNA methylation profiles of adipose tissue and weight loss before and after gastric bypass. This study provided a strong basis for future work and additional evidence for the role of DNA methylation in adipose tissue in obesity. The findings that the promoters of both the S100A8 and S100A9 genes were hypomethylated, in addition to their significantly increased expression in obese people, confirmed the validity of our findings.

There are a few limitations to this study. First of all, our data results only come from microarrays. Although it is clear that these two hub genes play a role in the pathogenesis of obesity, *in vitro* and *in vivo* studies are required to validate the relationship between these genes and obesity.

In summary, two GEO microarrays were explored for differential methylation and gene expression as epigenetic factors of obesity. After the functional analysis, we selected two DEMGs from another microarray data set (including 84

samples) for verification. Our results showed hypomethylation and up-regulation of S100A8 and S100A9 expression in obese patients. Also, correlation analysis showed that DNA methylation can regulate gene expression and lead to obesity.

Abbreviations

VAT: Visceral adipose tissue; DMPS: Different expression positions; DMGS: Differentially methylated genes; MetS: Metabolic syndrome; T2DM: Type 2 diabetes;

CVD: Cardiovascular disease; EWAS: Extensive epigenomic association studies;

BMI: Body mass index; DEGS: Differential expression genes; GO: Gene ontology;

KEGG: Kyoto Encyclopedia of Genes and Genomes; DO: Disease Ontology;

PPI: Protein-protein interaction; MCODE: Molecular complex detection;

RYGB: Roux-en-Y gastric bypass; GEO: Gene Expression Omnibus

Declarations

Acknowledgments

The authors would like to acknowledge all the participants of this study.

Author's contributions

N.-Y.C. conceived the study, participated in the design, undertook genotyping, performed the statistical analyses, and drafted the manuscript. L.M. participated in the design and analyzed the bioinformatics results. W.L. and D.-H.Z. conceived the study, and helped to draft the manuscript. L.H., J.H. and W.-X.S. collaborated to the genotyping. L.-L.L., Y.-X.L. and H.L. carried out the epidemiological survey and collected the samples. S.-L.P. and J.-H.P. supervised the whole work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish this article in the journal of Molecular Medicine.

Competing interests

The authors have no potential conflicts of interest to report.

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Figures

Figure 1

Normalization of all samples. (A) Before normalization; (B) After normalization.

Figure 2

Heatmap and volcano plots of DEGs and DMPs. (A) Heatmap for DEGs. Obesity groups are in the red cluster, and normal samples are in the green cluster. (B) Volcano plot for DEGs. The two vertical lines are the 2-fold change boundaries and the horizontal line is the statistical significance boundary ($P < 0.05$). Red dots show up-regulated genes, and green dots are down-regulated genes. (C) Heatmap for DMPs. Obesity groups are in the green cluster, and normals are in the blue cluster. (D) Volcano plot for DMPs. The vertical lines are the 0.05-fold change boundaries and the horizontal line is the statistical significance boundary ($P < 0.05$). Up-regulated DMPs are marked with red dots, and down-regulated are marked with green dots.

Figure 3

Venn Diagram showing the intersection of DEGs and DMGs.

Figure 4

Chromosome distribution of differentially methylated intergenic CpGs. Plot showing the distribution of differential intergenic CpG sites on 22 autosomes, and the X, and the Y chromosomes. Red is the hypermethylated region, and blue is the hypomethylated region with logFC values of the M value between obesity patients and healthy controls.

Figure 5

Functional enrichment of DEGs. The x-axis shows the ratio number of genes and the y-axis shows the pathway terms. The $-\log_{10}$ (P-value) of each term is colored according to the legend. (A) Gene Ontology; (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis; (C) Disease Ontology

Figure 6

Protein-protein interaction network analysis. (A) Protein-protein interaction network of the module genes. Edge stands for the interaction between two genes. A degree was used for describing the importance of protein nodes in the network, darkness red shows a high degree and light presents a low degree. (B - E) The significant modules identified from the protein-protein interaction network using the molecular complex detection method with a score of > 8.0. MCODEB score = 22.166, MCODEc score = 17.442, MCODED score = 9.278 and MCODEE score = 8.963.

Figure 7

Correlation analysis of DNA methylation and mRNA expression.

Figure 8

Verification of mRNA expression between obese and healthy samples in GSE109597.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable3.csv](#)
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