Original Paper

Long Non-Coding RNA Expression Profiling in Obesity Mice with Folic Acid Supplement

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Key Words

LncRNAs • Expression profile • Gene • Ontology • Pathway • Co-expression

Abstract

Background/Aims: Obesity is a major contributor to the growing prevalence of metabolic and cardiovascular diseases. This study was designed to investigate the effect of folic acid (FA) on obese mice by detecting the genome-wide expression profile of lncRNAs and mRNAs in the heart. **Methods:** Heart samples were collected from mice fed with standard diet (SD), high-fat diet (HFD) and high-fat diet with FA intake (HFDF). LncRNAs and mRNAs between HFD and HFDF group were analyzed by lncRNA microarray. Nine lncRNAs and mRNAs were validated using quantitative reverse transcription polymerase chain reaction (qRT-PCR). Bioinformatics prediction was used to investigate the potential function of these differentially expressed lncRNAs. Co-expression analysis was used to determine the transcriptional regulatory relationship of differentially expressed lncRNAs and mRNAs between two groups. **Results:** The expression of 58,952 lncRNAs and 20,145 mRNAs in HFD and HFDF groups was profiled by using microarrays. Gene Ontology and pathway analyses indicated that the biological functions of differentially expressed mRNAs were related to inflammation, energy metabolism, and cell differentiation. Co-expression networks composed of lncRNAs and mRNAs were also constructed to investigate the potential regulatory roles of differentially expressed lncRNAs on mRNAs. LncRNAs, namely, NONMMUT033847, NONMMUT070811, and NONMMUT015327, were validated through qRT-PCR, and these lncRNAs may be important factors regulating inflammation, energy metabolism, and cell differentiation. The expression levels of Dnajb1, Egr2, Hba-a1, Il1 β , Cxcl2, and Tnfsf9 were significantly different between HFD and HFDF. **Conclusions:** Results suggested that FA may improve the cardiovascular function of obesity and contribute to those lncRNAs associated with inflammation and cell differentiation. In a nutshell, the present study identified a panel of lncRNAs and mRNAs that may be potential biomarkers or drug targets relevant to the high-fat diet related obesity.

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Introduction

Recent obesity epidemic is a major contributor to the increasing prevalence of metabolic and cardiovascular diseases [1]. In many chronic inflammatory diseases, genetic and environmental factors are essential for the development of obesity and its associated diseases [2, 3]. Folic acid (FA) supplement can effectively reduce the homocysteine levels and insulin resistance of obese children and consequently prevent obesity-related complications, including cardiovascular and metabolic disorders [4]. Food fortified with FA also helps decrease the birth prevalence of specific congenital heart defects (CHDs) [5].

The overexpression of interferon beta, an immune-modulating cytokine, likely attenuates high-fat diet (HFD)-induced adipose inflammation and protect animals against obesity development [6]. Eicosapentaenoic acid (EPA), an omega-3 fatty acid, improves the regenerative capacity of mouse skeletal muscle cells exposed to saturated fat and inflammation [7]. Therefore, obesity is associated with chronic low-grade inflammation, and inflammatory responses can induce detrimental metabolic effects. In obesity, chronic low-grade inflammation occurs in many organs, including, but not limited to, white adipose tissue (WAT), brown adipose tissue, pancreas, liver, brain, muscle, and intestines [8] [9].

Genome-wide transcriptional studies have indicated that approximately 1% of the human genome produces biologically significant RNA transcripts, whereas a much larger proportion of the genome is transcribed into non-coding RNAs (ncRNAs) [10,] [11], which are non-protein-coding transcripts longer than 200 nucleotides [12] and are possibly involved in the regulation of various molecular and cellular functions. Long non-coding RNAs (lncRNAs) are widely distributed in the human genome. More than 90% of the human genome is composed of non-coding RNAs [13]. Changes in the amount of lncRNAs can induce aberrant gene expression that contributes to various disease states and biological functions [14]. Thus far, 5,446 lncRNA genes have been identified in the human genome. However, the functions of these genes remain unclear. Therefore, the co-expression of lncRNAs and mRNA should be analyzed to help predict their functional roles in disease development as a foundation for further functional and mechanistic studies. lncRNAs common to white and brown fat are essential for adipogenesis [15]. For instance, lnc-RAP1 (Firre) is exclusively nuclear and interactive with the nuclear matrix factor hnRNP U to mediate transchromosomal interactions between loci encoding adipogenic factors [16]. Differentially expressed circulating lncRNAs and mRNAs have been identified through microarray analysis between obese and non-obese human subjects [17]. Nevertheless, lncRNA biomarkers have yet to be identified in the heart of an obese individual.

In this study, the expression patterns of lncRNAs and mRNAs in the heart of mice fed with HFDF and HFD were demonstrated and validated through quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Gene-lncRNA co-expression networks were further constructed to determine interaction patterns among genes with their related co-expressed lncRNAs. Our results suggested that FA might significantly affect the cardiovascular function of obesity.

Materials and Methods

Ethics Statement

Male wild-type (WT) mice in a C57BL/6 background were bred and maintained in the Laboratory of Animal Experiments at the Capital Institute of Pediatrics, Beijing China. The study was approved by the Ethical Committee for the Capital Institute of Pediatrics.

Animal Models

C57BL/6 background mice were purchased from HFK Bioscience Company. All of the mice used in the experiment were within 3 weeks old, maintained under specific pathogen-free conditions, and handled according to the animal welfare regulations of the Capital Institute of Pediatrics. Experimental procedures

were approved by the Animal Subjects Committee of the Capital Institute of Pediatrics. Male mice were randomly divided into three treatment groups (n=15 each): WT with HFD feeding and WT with HFDF feeding compared with WT with SD feeding. The mice were fed for 12 weeks. In this procedure, 20 mg of FA was dissolved in 1 kg of water. The body weight of each group of mice was monitored weekly. For intraperitoneal glucose tolerance testing, mice were fasted overnight and then subjected to intraperitoneal injection with glucose (2.0g kg⁻¹ of body weight) at the end of the experiment. Venous blood was subsequently drawn by tail clipping at 0, 15, 30, 60, 120 min. Blood glucose levels were measured with Accu-Chek glucometers (Roche Applied Science, Penzberg, Germany). Plasma cytokine and adipokine levels were measured using Milliplex Mouse Serum Cytokine and Adipokine Kits (Millipore Corp, Billerica, MA) on the Luminex IS 100 system (Luminex Corp, Austin, TX), as per the manufacturer's instructions.

Preparation of array hybridization

SBC 4x180K mouse lncRNA microarrays were custom-designed by using Agilent eArray according to the manufacturer's recommendations (<https://earray.chem.agilent.com/earray>). The microarray contained 20,145 mRNA probes and 58,952 lncRNA probes. The lncRNA sequence was derived from four databases, namely GENCODE v21, Ensembl, Noncode v4, and UCSC. After the labeled cRNAs were purified, each slide was hybridized and washed according to the manufacturer's instructions (Agilent Technologies). Raw data were normalized with quantile algorithm and limma packages in R. Differentially expressed lncRNAs and mRNAs were identified in terms of p-value and fold change. Experiments and analyses were performed by Shanghai Biotechnology Corporation.

Quantitative real-time PCR

Total RNA was extracted from mice hearts using TRIzol reagent (Invitrogen, USA) and then reverse-transcribed with a high-capacity RNA-to-cDNA kit (Invitrogen, USA) according to the manufacturer's recommendations. The expression of significantly different lncRNAs and mRNAs in all of the subjects included in this study was determined through real-time PCR with SYBR Select Master Mix (Invitrogen, USA), and GAPDH was used as an internal control. For quantitative results, the expression of each lncRNA was represented as fold change by using 2- $\Delta\Delta C_t$ methods. Differences in the lncRNA expression between HFDF and HFD were analyzed with Student's t-test. $P < 0.05$ was considered significant. The primers used in this study are listed in Table 1.

Functional group analysis

Gene Ontology (GO) terms were searched from GO Database (<http://www.geneontology.org/>). P-values for differentially expressed genes in all of the GO categories were calculated, and the threshold of significance was defined as $P < 0.05$. Differentially expressed mRNAs were subjected to pathway analysis through KEGG (<http://www.genome.jp/kegg/>). Signaling pathways were analyzed on the basis of significance level with $P < 0.05$ as the threshold.

Co-expression network

Gene co-expression networks were constructed according to the normalized signal intensity of individual genes to identify interactions among genes and lncRNAs [29]. Data were preprocessed by considering the median gene expression value of all transcripts expressed from the same coding gene but without providing a special treatment of the lncRNA expression value. These data were then screened for differentially expressed lncRNAs and mRNAs and removed from the dataset. For each analyzed pair of

Table 1. Verification of gene expression changes by qRT-PCR. Primers used in this paper

Primer Name	Sequence (5' to 3')	Lengths (bp)
NONMMUT033847	Fw AGCAGGGCAGGCTACTCACA	171
	Rev GCCACCGTACCATGAAGCAC	
NONMMUT070811	Fw GAGGGGTACTTGGCTGTGGA	190
	Rev GCTACGAACGGTTTCAGAATTTGT	
NONMMUT015327.2	Fw CAGTGTGGGAGCATGTGAAC	138
	Rev TCCGAGATGCCTCTACTCAAGAC	
Dnajb1	Fw GGACGAGACCTCGAACAACATT	222
	Rev GGGACTTTCGCCGCAT	
Egr2	Fw CGGTGACCATCTTCCCAAT	138
	Rev GAGCGAAGCTACTCGGATACG	
Hba-a1	Fw CCTCATTGTGATGAAGCCACG	272
	Rev GTGCTCACAGAGGAAGGAAT	
Tnfsf9	Fw AGGAGGAGAGAAAGTTCCG	214
	Rev GTAGTCTGCTCCATGAAGC	
Cxcl2	Fw GTAGTTGGAAGGTGATGCATTT	224
	Rev CCCTGCTGACAATAAGACAGAG	
IL-1 β	Fw GTTCTCTGAGAAATGGGAGC	143
	Rev CTGGTCATCATCAGAGAAGG	
Actin	Fw CGTTGACATCCGTAAGACCTC	159
	Rev ACAGAGTACTTGCGCTCAGGAG	

genes, Pearson correlation was calculated and pairs (only lncRNA-mRNA) with significant correlations were selected to construct the network [30]. The strongest correlations (0.99 or greater) were included in these renderings to prepare a visual representation. In this representation, each gene corresponded to a node and, a strong correlation, either positive or negative, was determined when two genes were connected by an edge. Co-expression networks were obtained by using Cytoscape. The degree and normalized degree were calculated to examine the topological property of this graph. A degree was defined as the number of directly linked neighbors [18]. The normalized degree was calculated by dividing the maximum degree in the same network to reduce the difference between individuals.

Statistical Analysis

Data were expressed as mean $\pm$ standard error of mean. Differences between groups were initially analyzed with Student's t-test or ANOVA and then with Newman-Keuls multiple comparison test by using GraphPad (GraphPad Prism version 5.0 for Windows, GraphPad). A value of $P < 0.05$ was considered statistically significant.

Results

Body weight, glucose tolerance and plasma adipokine levels of the three mouse groups

After 12 weeks of treatment, changes in body weight between the HFD and HFDF groups were closely similar. And the body weight of HFD group were significant increased than that of SD group since 3 weeks of treatment ($P < 0.05$, Fig. 1A). However, glucose tolerance test were significant improved, indicative of improved glucose tolerance ($P < 0.05$, Fig. 1B). Plasma leptin levels in the HFD group were remarkably higher than those in the HFDF group, and no significant differences in insulin, resistin, and PAI-1 ($P < 0.05$, Fig. 1C).

Differentially expressed lncRNAs

A genome-wide analysis of lncRNA and mRNA expression was performed to profile differentially expressed lncRNAs and mRNAs in FA intake and matching obesity. Hierarchical clustering was analyzed according to the lncRNAs and mRNAs expression values in the microarray (Fig. 2). The microarray data revealed that 220 genes were differentially expressed between the two groups. Of these genes, 122 lncRNAs were up-regulated and 98 lncRNAs were down-regulated (fold change ≥ 1.5 , $P < 0.05$). Hierarchical clustering analysis was conducted to arrange specimens into groups according to their expression levels, and the top 20 differentially expressed lncRNAs are listed in Table 2.

Differentially expressed mRNAs

A total of 101 mRNAs were differentially expressed between the two groups by using the same criteria of lncRNAs. Of these genes, 88 mRNAs were up-regulated and 13 mRNAs

Fig. 1. The body weight, glucose tolerance testing and serum adipokine levels in SD, HFD and HFDF group. (A) the body weight changes in standard diet, high-fat diet or high-fat diet plus FA intake fed mice during 12-week treatment. (B) glucose tolerance testing and (C) the serum adipokine levels are tested at the end of experiment. Data are presented as means $\pm$ SEM, $n = 15$. * $P < 0.05$, ** $P < 0.01$ vs HFD group.

Fig. 2. Profiles of differentially expressed genes in HFDF compared to HFD group. (A) Differentially expressed lncRNAs and (B) differentially expressed mRNAs were subjected to hierarchical clustering. Red color indicates high relative expression and green color indicates low relative expression. $P < 0.05$, $FC \geq 2$.

Table 2. Top 20 significantly differentially expressed lncRNAs

RNA Accession	Gene Symbol	Chr	Start	End	P values	Fold change
NR_038032	Gm15663	chr10	105574550	105583870	0.000973306	1.899707836
NONMMUT059061	NONMMUG036701	chr6	128964191	128967252	0.001433567	2.151778543
NR_045328	A330009N23Rik	chr15	101221190	101225186	0.001853522	1.657590951
NONMMUT000870	NONMMUG000588	chr1	52185063	52186843	0.002636463	1.555908649
NONMMUT051427	NONMMUG031897	chr5	21951858	21952499	0.003097323	2.002607194
NONMMUT029386	NONMMUG018157	chr17	34981862	34983981	0.003473757	1.551636372
NONMMUT050450	NONMMUG031254	chr4	144131848	144133881	0.00351878	2.005182058
NONMMUT065604	NONMMUG040577	chr8	49706193	49707681	0.004088748	2.111843634
NONMMUT020986	NONMMUG013027	chr14	55481590	55484028	0.004141571	0.652487258
NONMMUT036501	NONMMUG022457	chr2	29496511	29500548	0.004230885	0.652489439
NONMMUT052591	NONMMUG032652	chr5	67260340	67261262	0.004268772	2.269280861
ENSMUST00000104403	ENSMUSG00000077591	chr10	45268603	45268731	0.004354266	3.081275478
ENSMUST00000145794	ENSMUSG00000085282	chr10	105574559	105583874	0.0051675	1.587201826
NONMMUT005687	NONMMUG003666	chr10	56971989	56982231	0.005868971	1.710593567
NONMMUT037692	NONMMUG023266	chr2	68135664	68136612	0.005954769	1.995954743
NONMMUT053714	NONMMUG033329	chr5	109874406	109875259	0.007239241	0.601696143
NR_102275	AI839979	chr5	31569591	31571397	0.007472296	2.104869272
NONMMUT050442	NONMMUG031247	chr4	143685016	143687052	0.007498551	1.949747508
NONMMUT048568	NONMMUG030140	chr4	108052367	108055221	0.007836593	0.644541465
ENSMUST00000192441	ENSMUSG00000102950	chr1	181986336	181990204	0.008368272	1.556936786

were down-regulated (fold change ≥ 1.5 , $P < 0.05$). The first 20 differentially expressed mRNAs are listed in Table 3.

Function analysis of differentially expressed genes

Differentially expressed mRNAs were subjected to GO enrichment analysis to identify GOs with higher confidence. Enrichment indicates the significance of a specific function. As enrichment increases, the corresponding function is more specific and thus helps identify GOs with a more concrete functional description in experiments. These functional analyses were performed for all of the differentially expressed mRNAs. GOs associated with differentially expressed transcripts were “negative regulation of transcription from RNA polymerase II promoter,” “multicellular organismal development,” and “transport.” Gene ontology analysis which primarily related to transport, cell development, metabolic process are shown in Fig. 3A.

Table 3. Top 20 significantly differentially expressed mRNAs

RNA Accession	Gene Symbol	Gene Description	Chr	Start	End	P values	Fold change
NM_020275	Tnfrsf10b	tumor necrosis factor receptor superfamily%2C member 10b	chr14	69767471	69784411	0.000760301	1.630787619
NM_026232	Slc25a30	solute carrier family 25%2C member 30	chr14	75761998	75787037	0.001119217	1.520735231
NM_080510	Trim69	tripartite motif-containing 69	chr2	122160699	122179027	0.004089418	2.484901038
NM_01011861	Olfir331	olfactory receptor 331	chr11	58501597	58502554	0.004497991	1.602501561
NM_138952	Ripk2	receptor (TNFRSF)-interacting serine-threonine kinase 2	chr4	16123374	16163498	0.005140857	1.541011315
NM_029363	2010109A12Rik	RIKEN cDNA 2010109A12 gene	chr5	93206517	93213474	0.005303734	1.623702768
NM_175493	Gpr68	G protein-coupled receptor 68%2C transcript variant 1	chr12	100876681	100908198	0.006847167	2.364599468
NM_008690	Nrkbe	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor%2C epsilon	chr17	45555699	45563168	0.006930452	1.823944579
NM_001033156	Fbxo33	F-box protein 33	chr12	59200654	59219483	0.007867056	1.518140761
NM_138630	Arhgap4	Rho GTPase activating protein 4%2C transcript variant 1	chrX	73894351	73911298	0.008836336	1.551066197
NM_001287176	Alpl	alkaline phosphatase%2C liver/bone/kidney%2C transcript variant 3	chr4	137741730	137769820	0.008869479	1.519689763
NM_001270722	Gm21319	predicted gene%2C 21319	chr12	87772424	87775755	0.009050529	1.520749363
NM_010479	Hspa1a	heat shock protein 1A	chr17	34969358	34972156	0.009735401	8.220655127
NM_028657	Mfsd12	major facilitator superfamily domain containing 12	chr10	81357569	81364035	0.009809235	1.585460094
NM_018808	Dnajb1	DnaJ (Hsp40) homolog%2C subfamily B%2C member 1	chr8	83608174	83611903	0.009822239	2.799157241
NM_001291066	Adam8	a disintegrin and metalloproteinase domain 8%2C transcript variant 2	chr7	139978937	139992509	0.009862383	1.578144923
NM_009992	Cyp1a1	cytochrome P450%2C family 1%2C subfamily a%2C polypeptide 1%2C transcript variant 1	chr9	57687927	57703824	0.010123204	1.747740192
NM_001122640	Arhgap17	Rho GTPase activating protein 17%2C transcript variant 2	chr7	123279148	123369915	0.010274411	1.616606218
NM_172462	Zfp11	zinc finger protein 11	chr5	129654594	129670088	0.010850417	1.633307005
NM_026209	Saysd1	SAYSVFN motif domain containing 1	chr14	20075635	20083172	0.011085833	1.952614937

Fig. 3. Gene ontology analysis and pathway analysis of differentially expressed unigenes. (A) The top 10 gene ontology that related to transport, cell development, metabolic process analysis of differentially expressed unigenes. (B) The top 10 pathway which related to inflammation, metabolism and differentiation analysis of all differentially expressed unigenes.

Pathway analysis indicated that 36 pathways were significantly enriched among the differentially expressed transcripts. Many of these pathways, such as “Cytokine-cytokine receptor interaction pathway,” “Osteoclast differentiation pathway,” “MAPK signaling pathway,” “Metabolic pathways,” and “Folate biosynthesis,” were linked to inflammation, energy metabolism, and cell differentiation. The pathways which related to inflammation, metabolism and differentiation are shown in Fig. 3B.

Co-expression analysis between differentially expressed lncRNAs and coding genes in HFD and HFDF

Pearson correlation was calculated and general coding-noncoding gene co-expression networks were constructed in each of the HFD and HFDF groups with the differentially expressed lncRNAs and mRNAs from this study to determine the transcriptional regulatory relationship. A total of 432 co-expression edges connecting 136 nodes, with 91 coding

Table 4. Top 15 lncRNAs and mRNAs with largest degree differences in HFDF co-expression network compared with HFD groups

Gene	HFD egree	HFDF Degree	abs_network_diffence	style	regulation
Dnajb1	6	0	0.75	coding	up
NONMMUT033847	2	9	0.75	nocoding	down
NONMMUT070811	0	6	0.666667	nocoding	down
NONMMUT015327	7	2	0.652778	nocoding	down
Egr2	6	1	0.638889	coding	up
ENSMUST00000124376	3	9	0.625	nocoding	up
Gem	7	3	0.541667	coding	up
Hba-a1	7	3	0.541667	coding	down
Gm14548	2	7	0.527778	coding	up
NONMMUT004166	5	1	0.513889	nocoding	down
NONMMUT022094	3	8	0.513889	nocoding	up
Tectb	5	1	0.513889	coding	up
Cd300lb	4	0	0.5	coding	up
Gm13288	4	0	0.5	coding	up
Spaca3	4	0	0.5	coding	up

Fig. 4. Coding-no-coding gene co-expression network was constructed based on the correlation analysis between the differentially expressed lncRNAs and mRNAs between (A) HFD and (B) HFDF group. In the network, Genes colored in red are up-regulated, genes colored in green are down-regulated and genes denoted a blue ring encode lncRNAs. Grey solid lines indicate correlation of genes. Node size represents the node degrees.

genes and 45 lncRNAs, were found in the FA intake network (Fig. 4B), whereas 464 co-expression edges connecting 135 nodes, with 89 coding genes and 46 lncRNAs, were found in the control obesity network (Fig. 4A). According to the degree analysis, Dnajb1, Egr2, Hba-a1, NONMMUT033847, NONMMUT070811, and NONMMUT015327 yielded the largest

Fig. 5. lncRNAs and mRNAs in HFD and HFDF of mice hearts were validated by qRT-PCR. (A) 6 lncRNAs and mRNAs which analyzed by co-expression were validated by qRT-PCR. (B) 3 inflammatory genes were validated by qRT-PCR. The height of the columns in the figure represents the expression level relative to GAPDH. The data are expressed as mean SEM. * $P < 0.05$, ** $P < 0.01$ vs HFD group.

changes in the degree within the network. The first 15 lncRNAs and mRNAs with the largest changes in the degree between HFD and HFDF are shown in Table 4. These genes primarily participate in cytokine–cytokine receptor interaction, osteoclast differentiation, MAPK signaling pathway, Jak-STAT signaling pathway, neurotrophin signaling pathway, and folate biosynthesis. These results indicate that the lncRNAs in the network likely perform similar functions and thus might play a vital role in the molecular regulation of obesity.

QRT-PCR validation

NONMMUT033847, NONMMUT070811, NONMMUT015327, Egr2, Dnajb1, and Hba-a1 were chosen to validate our microarray results (Fig. 5A). The relative genes involved in the cytokine–cytokine receptor interaction pathway (Il1 β , Cxcl2, and Tnfsf9) were also detected through qRT-PCR (Fig. 5B). QRT-PCR results are consistent with microarray findings.

Discussion

In this study, the genome-wide expression profile of lncRNAs and mRNAs in the heart of mice fed with SD and HFD were investigated through microarray. A total of 220 lncRNAs, including 122 up-regulated and 98 down-regulated were identified to be differentially expressed between the two groups of mice. Simultaneously, 101 differentially expressed protein coding mRNAs from the same chip were also identified, including 88 up-regulated and 13 down-regulated mRNAs. The differences in the expression of lncRNAs and mRNAs detected through qRT-PCR were consistent with those observed through microarray.

Cardio-metabolic diseases are characterized as a combination of multiple risk factors of cardiovascular diseases (CVD) and metabolic diseases, including diabetes mellitus, dyslipidemia, hypertension, and abdominal obesity [19]. Childhood obesity is a chronic and complex metabolic disorder, which is associated with a high risk of various medical and psychosocial consequences [20]. CVD and type 2 diabetes mellitus are the most common preventable complications of childhood obesity. The presence of biochemical indicators, such as insulin resistance, hyperinsulinemia, and hyperhomocysteinemia, are associated with the early phase of these conditions, and proper interventions can prevent the development of complications [21, 22].

The role of FA in metabolic health has been reported. Silva et al. revealed that FA deficiencies are found more frequently among individuals subjected to gastric bypass than gastric banding of bariatric techniques [23]. In an urban low-income prospective birth cohort, maternal plasma folate concentrations exhibit an L-shaped association with child overweight or obesity sufficient folate concentrations provide beneficial effects on individuals, especially obese mothers [24]. Dehkordi demonstrated that FA in two examined doses can be a safe and effective supplement for obese children to reduce homocysteine levels and insulin resistance; consequently, FA can prevent obesity-related complications, including cardiovascular and metabolic disorders [4]. The homocysteine-reducing effects of FA supplementation are observed in obese children [25]. Inflammation is increased in

rats fed with high-fat-adequate FA (HF-AFA), and this increase is associated with impaired glucose tolerance compared with HF-EFA-fed rats. FA induces the PPAR γ expression and triglyceride accumulation in 3T3-L1 cells [26]. FA may influence the pathogenesis of CHDs through other more complex pathways, including epigenetic mechanisms responsible for transgenerational effects [27]. Epigenetic mechanisms are possibly implicated in this process because the beneficial effects of FA fortification in reducing the prevalence of CHDs at birth may require more than one generation to become fully apparent [27]. In a Hungarian randomized trial, the birth prevalence of congenital cardiovascular anomalies is lower among women prescribed with periconceptional multivitamins, including FA, and trace element supplementation than among women given with supplemental trace elements only [28]. Our results demonstrated that NONMMUT033847, NONMMUT070811, and NONMMUT015327 were down-regulated in the HFDF group. QRT-PCR results validated our microarray data, which indicated that identified lncRNAs might play an important role in improving the cardiovascular functions during obesity.

Considering the importance of the cytokine–cytokine receptor interaction pathway, we focused on *Csf2rb*, *Cxcl2*, *Eda2r*, *Il10ra*, *Il1b*, *Lif*, *Tnfrsf10b*, and *Tnfsf9*. These genes are involved in the regulation of cell development, activation, growth, and motility. Nehete et al. observed the plasma-circulating levels of pro-inflammatory Th-1 cytokines, interferon gamma, interleukin-6, interleukin-12p40, tumor necrosis factor, soluble CD40 ligand, and interleukin-1b, and those of anti-inflammatory Th-2 cytokines, interleukin-4, interleukin-RA, interleukin-10, and interleukin-13 increase in overweight and obese chimpanzees [29]. In cytokines and chemokines in WBCs, TNF-SF5, 7, and 9 and CCL 8 and 10 are significantly higher in children with type 1 diabetes than in healthy children [30]. Chemokine ligand-2 (CXCL2), a WAT, produces up-regulated chemokines in obesity. Activated neutrophils in obesity may influence the functions of visceral WAT endothelial cells and contribute to WAT inflammation [31]. Our PCR results indicated that *Il1b*, *CXCL2*, and *Tnfsf9* are significantly higher in the HFDF group than in the HFD group. *Hba-a1* was down-regulated and *Dnajb1* and *Egr2* were up-regulated in the HFDF group. QRT-PCR results validated our microarray data.

This study was limited by the following. (i) GO and pathway analyses are required to further investigate the relationships among noncoding RNAs, coding RNAs, and proteins. (ii) lncRNAs and mRNAs validated by qPCR should be further tested on cell level.

In summary, this study identified the expression patterns of lncRNAs and mRNAs in the heart of mice fed with HFD and HFDF. lncRNAs were detected as a potential regulatory factor of obesity most likely through inflammation, energy metabolism, and cell differentiation. Further work should be performed to understand the biological functions and molecular mechanisms of specific lncRNAs implicated in obesity.

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Disclosure Statement

None of the authors have any potential conflicts of interest associated with this research.

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