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Dysregulation of long non-coding RNA ZFAS1 in children with obesity and its predictive value for metabolic syndrome

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Abstract

Introduction: The purpose of this study was to analyse the correlation between zinc finger antisense 1 (ZFAS1) and obesity and the diagnostic value of obesity complicated with metabolic syndrome (obesity-MS).

Material and methods: Serum levels of ZFAS1 were measured by quantitative real-time polymerase chain reaction (qRT-PCR) in healthy children, children with simple obesity, and children with obesity-MS. The diagnostic accuracy of ZFAS1 was evaluated using the receiver operator characteristic (ROC) curve. Pearson's method was used to study the correlation between ZFAS1 and other indicators. Logistic regression was used to analyse the significance of ZFAS1 in the progression of obesity to obesity-MS. StarBase V2.0 was used to predict the target gene of ZFAS1 (miR-193a-3p). Bioinformatics methods were used to identify the molecular functions and possible enrichment signalling pathways of downstream target genes of miR-193a-3p.

Results: The expression of ZFAS1 in patients with obesity and obesity-MS showed a gradual upward trend, while the expression of miR-193a-3p was the opposite. ZFAS1 could identify obesity-MS children from children with obesity (area under the curve [AUC] = 0.880). ZFAS1 was significantly correlated with body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP), and other indicators, while ZFAS1 was an independent influencing factor for the development of obesity into obesity-MS. Furthermore, a total of 104 downstream target genes of miR-193a-3p were identified, which participated in many biological processes such as protein phosphatase regulation, activation of transcription factor activity, and enrichment in MAPK signalling pathway.

Conclusion: ZFAS1 is dysregulated in obesity and obesity-MS. Abnormal expression of ZFAS1 has high diagnostic value for obesity-MS, and it has the potential to become a clinical diagnostic biomarker for obesity-MS.

Key words: *ZFAS1; obesity; metabolic syndrome; miR-193a-3p*

Introduction

The prevalence of overweight and obesity in children is increasing year by year [1, 2]. Obesity refers to the abnormal accumulation of fat in the body tissues due to long-term excessive intake of calories and lack of exercise, which eventually leads to the secretion of adipokine, resulting in disorder of systemic glucose and lipid metabolism and inflammatory response [3, 4]. Metabolic syndrome (MS) comprises a group of clinical syndromes characterised by hyperglycaemia, hypertension, and dyslipidaemia on the basis of obesity, and elevated body mass index (BMI) is an independent risk factor for the development of MS [5, 6]. Obesity and MS can induce systemic complications such as type 2 diabetes (T2D), non-alcoholic fatty liver disease, cardiovascular disease, and polycystic ovary syndrome (PCOS). At present, the main treatment for obesity and MS in children are symptomatic treatment, including exercise and diet intervention, drug therapy, and weight loss surgery. Among them, the drug treatment of childhood obesity is not effective, and there is a lack of long-term drug safety information. Therefore, when dealing with obesity and its complications, it is essential to understand the pathological process and pathogenic mechanism to have a clearer understanding of the disease, which is conducive to the diagnosis and treatment of the disease.

Long non-coding RNA (lncRNA) is a regulatory RNA with a length of more than 200 nucleotides but lacks the protein coding potential. Several lncRNAs have been proven to have positive or negative functions in regulating fat synthesis. One study showed that the gut microbiota reprogrammed intestinal

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lipid metabolism by inhibiting the expression of small nucleolar RNA host gene 9 (SNHG9) in small intestinal epithelial cells [7]. Another study found that knocking out nuclear-enriched abundant transcript 1 (NEAT1) can inhibit lipid accumulation in nonalcoholic fatty liver disease [8]. Zinc finger antisense 1 (ZFAS1) is a gene located on human chromosome 20q13.13, which is dysregulated in many human tumours, such as liver cancer, ovarian cancer, and breast cancer [9–11]. Recently, the role of ZFAS1 in metabolic diseases has been widely reported. For example, ZFAS1 is upregulated in patients with nonalcoholic fatty liver disease, and excessive ZFAS1 induces increased lipid deposition, enhanced oxidative stress, and unbalanced inflammatory response [12]. In a study on atherosclerosis, an increase of ZFAS1 directly led to inhibition of cholesterol efflux from macrophage-derived foam cells [13]. In addition, a study on the relationship between obesity and breast cancer showed that the reduction of ZFAS1 in normal breast tissue was negatively correlated with BMI [14]. So far, relatively few studies have linked ZFAS1 to obesity or metabolic syndrome.

In this study, by analysing the expression level of ZFAS1 in the serum of children with obesity and obesity-MS, the diagnostic value of ZFAS1 for obesity and obesity-MS was evaluated for the first time, and the influencing factors of these patients' development from obesity to obesity combined with MS was further evaluated.

Material and methods

Subjects and samples

This study was carried out after obtaining approval from the Ethics Committee of Taihe Hospital, Affiliated Hospital of Hubei University of Medicine. All study subjects and their legal guardians are aware of the study, and we obtained their written informed consent. All procedures are carried out with reference to the guidelines on human trials contained in the Declaration of Helsinki.

According to Centres for Disease Control and Prevention (CDC) criteria, 60 children with obesity aged 7–13 years were included in this study. The inclusion criteria for childhood obesity followed the criteria defined by the CDC as having a BMI value greater than or equal to the 95th percentile for children ages 2 to 19 years [15]. On the basis of the diagnosis of obesity, the following 2 or more points are considered as obesity complicated with metabolic syndrome (referred to as obesity-MS in this paper) [16]: 1) children with T2DM or fasting blood glucose (FBG) ≥ 5.6 mmol/L; 2) elevated blood pressure: systolic blood pressure (SBP) ≥ 130 mmHg and/or diastolic blood pressure (DBP) ≥ 85 mmHg; 3) high-density lipoprotein cholesterol (HDL-C) < 1.03 mmol/L; and 4) increased serum triglyceride (TG) ≥ 1.7 mmol/L. Any child who meets the criteria for obesity but does not meet the criteria for obesity-MS is classified as simple obesity. Exclusion criteria: 1) children with chronic diseases, such as neuroendocrine diseases, genetic diseases, and tumours; 2) children receiving drug therapy; and 3) children suffering from other acute and chronic infectious diseases. Another 45 healthy children were selected as the control group (Fig. 1).

General information: age, sex. Physical examination data: All subjects were required to take off their clothes and shoes for measurement. The height, weight, waist circumference (WC), SBPm and DBP were measured and evaluated by professional medical staff. BMI = weight/height^2 (kg/m^2). Laboratory data: FBG, total cholesterol, TG, HDL-C, low-density lipoprotein cholesterol (LDL-C), homeostasis model assessment-insulin resistance index (HOMA-IR), and other test indicators were measured by the laboratory department.

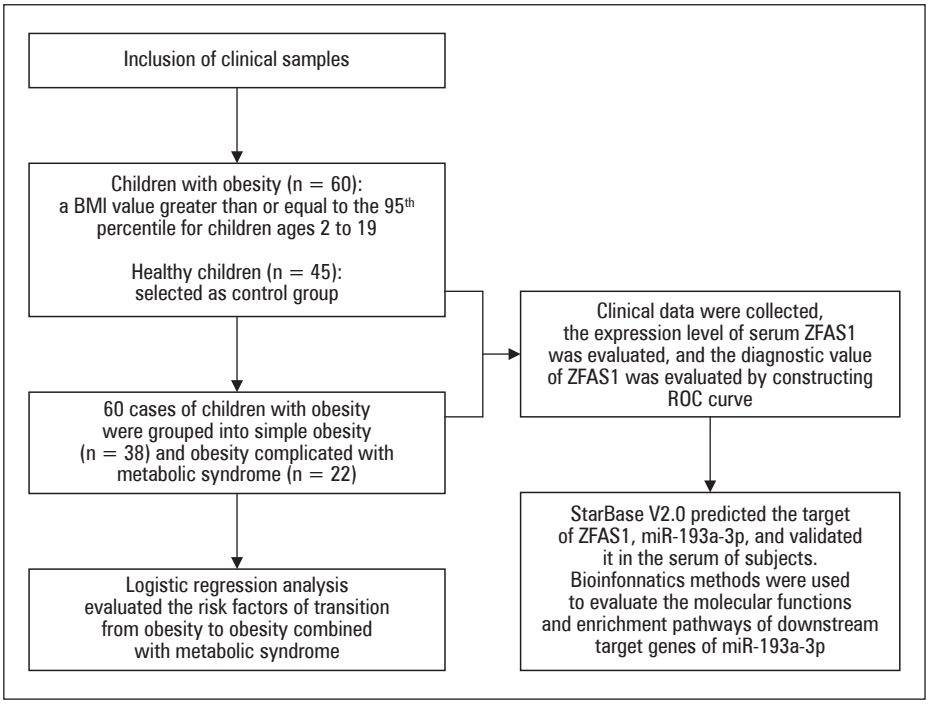

Figure 1. *The flowchart shows the basic idea and analysis method of the experimental research. BMI — body mass index; ZFAS1 — zinc finger antisense 1; ROC — receiver operator characteristic*

RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

ZFAS1 or miR-193a-3p in serum or cells were extracted by TRIzol reagent (Invitrogen, USA). After adding TRIzol reagent, the total RNA was extracted by chloroform, isopropyl alcohol, and anhydrous ethanol. Then, the concentration of RNA was measured by ultra-microspectrophotometer (IMPLEN, P330, Germany). When the value of OD260/280 was between 1.9 and 2.1, the purity of RNA was suitable for experiment. Next, according to the instructions of the reverse transcription kit, the reaction system was configured for reverse transcription to obtain cDNA. The cDNA was amplified according to the instructions of the SYBR Premix Ex Taq II kit (Takara, Dalian, China). Cycle threshold (Ct) values were recorded after amplification, and the relative expression level of ZFAS1 was calculated using the $2^{-\Delta\Delta Ct}$ method proposed by Livak and Schmittgen. The gene expression data of ZFAS1 and miR-193a-3p were normalised using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 as endogenous controls, respectively. The primers required for this experiment were as follows: ZFAS1: 5′-ACCAGGCTTTGATTGAAC-3′ (f), 5′-ATTCCATCGCCAGTTTCT-3′ (r); miR-193a-3p: 5′-CGCGAACTGGCCTACAAAGT-3′ (f); 5′-AGTGCAGGGTCCGAGGTATT-3′ (r); GAPDH: 5′-CTGCACCACCAACTGCTTAG-3′ (f); 5′-AGGTCCACCACTGACACGTT-3′ (r); U6: 5′-CTCGCTTCGGCAGCACA-3′ (f), 5′-AACGCTTCACGAATTTGCGT-3′ (r).

Bioinformatics analysis

The suspected complementary sites between miR-193a-3p and ZFAS1 were predicted by Starbase V2.0 (http://starbase.sysu.edu.cn/). Furthermore, the downstream target genes of miR-193a-3p were predicted by the TargetScan (http://www.targetscan.org), EVmiRNA (http://bioinfo.life.hust.edu.cn/EVmiRNA), and miRDB (https://mirdb.org/) databases. To verify the biological processes (BP), cellular components (CC), and molecular functions (MF) of these target genes, gene ontology (GO) analysis was performed. Meanwhile, Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis was used to analyse the possible enrichment signal pathways of these target genes. GO and KEGG enrichment analyses were carried out using the Bioinformatics online platform (http://www.bioinformatics.com.cn).

Statistical analysis

SPSS21.0 and GraphPad Prism 6.0 were used for statistical analysis. Continuous variables that conform to a normal distribution are expressed as mean ± standard deviation (SD). The t test was used to compare the continuous variables between 2 groups, and one-way analysis of variance (ANOVA) was used for comparison between multiple groups. The sex difference was compared by chi-square test. A receiver operator characteristic (ROC) curve was constructed to evaluate the diagnostic value of ZFAS1 in obesity. The correlation between ZFAS1 and clinical indicators was evaluated by Pearson method. Logistic regression was performed to estimate the relationship between each variable and the occurrence of obesity-MS. Obesity-MS was taken as the dependent variable (Assign: Obesity-MS = 1, Obesity = 0), and age (Assign: > 10 years = 1, ≤ 10 years = 0), sex (Assign: female = 1, male = 0), BMI (Assign: > 28.56 kg/m^2 = 1, ≤ 28.56 kg/m^2 = 0), WC (Assign: > 91.90 cm = 1, ≤ 91.90 cm = 0), SBP (Assign: > 113.4 mm Hg = 1, ≤ 113.4 mm Hg = 0), DBP (Assign: > 74.28 mm Hg = 1, ≤ 74.28 mm Hg = 0), FBG (Assign: > 4.57 mmol/L = 1, ≤ 4.57 mmol/L = 0), TC (Assign: > 4.25 mmol/L = 1, ≤ 4.25 mmol/L = 0), TG (Assign: > 1.32 mmol/L = 1, ≤ 1.32 mmol/L = 0), HDL-C (Assign: > 1.19 mmol/L = 1, ≤ 1.19 mmol/L = 0), LDL-C (Assign: > 2.37 mmol/L = 1, ≤ 2.37 mmol/L = 0), HOTAIR (Assign: > 5.28 = 1, ≤ 5.28 = 0), and the ZFAS1 level were classified as independent variables. $P < 0.05$ was considered as a significant difference.

Results

Comparison of subjects' general information and clinical indicators

The clinical data of healthy children and all children with obesity are summarised in Table 1. The results showed that there was no significant difference in age and sex between the 2 groups, which indicated that they were comparable. The levels of BMI, WC, SBP, DBP, TC, HDL-C, LDL-C, and HOMA-IR in children with obesity were significantly higher than those in healthy children ($p < 0.01$), while the levels of FBG and TC were not significantly different from those in healthy children

Table 1. *Comparison of clinical data between healthy children and children with obesity*

Items	Controls (n = 45)	Obesity (n = 60)	p
Age [years]	9.41 ± 2.06	9.56 ± 1.92	0.636
Sex [male/female]	21/24	27/33	0.534
BMI [kg/m^2]	18.24 ± 1.03	29.89 ± 2.84	< 0.001
WC [cm]	64.37 ± 2.35	93.13 ± 7.74	< 0.001
SBP [mmHg]	96.56 ± 5.18	114.57 ± 12.38	< 0.001
DBP [mmHg]	65.78 ± 3.17	77.53 ± 8.77	< 0.001
FBG [mmol/L]	4.99 ± 0.29	4.85 ± 0.52	0.274
TC [mmol/L]	4.24 ± 0.52	4.33 ± 0.55	0.742
TG [mmol/L]	0.58 ± 0.13	1.51 ± 0.89	< 0.001
HDL-C [mmol/L]	1.43 ± 0.19	1.24 ± 0.25	0.001
LDL-C [mmol/L]	1.97 ± 0.37	2.36 ± 0.73	0.003
HOMA-IR	1.71 ± 0.81	5.33 ± 3.27	< 0.001

BMI — body mass index; WC — waist circumference; SBP — systolic blood pressure; DBP — diastolic blood pressure; FBG — fasting blood glucose; TC — total cholesterol; TG — triglycerides; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; HOMA-IR — homeostasis model assessment-insulin resistance index. Data are presented as mean ± standard deviation (SD); p < 0.05 means significant difference

Table 2. *Comparison of clinical data between children with obesity and obesity complicated with metabolic syndrome (Obesity-MS)*

Items	Simple obesity (n = 38]	Obesity-MS (n = 22]	p
Age [years]	9.56 ± 1.79	9.23 ± 2.14	0.501
Sex [male/female]	17/21	10/12	0.726
BMI [kg/m^2]	27.67 ± 1.88	30.43 ± 3.57	0.002
WC [cm]	87.29 ± 6.45	97.89 ± 4.06	< 0.001
SBP [mmHg]	109.63 ± 10.03	120.85 ± 12.77	< 0.001
DBP [mmHg]	76.18 ± 9.26	78.55 ± 8.93	0.078
FBG [mmol/L]	4.55 ± 0.43	5.13 ± 0.78	0.004
TC [mmol/L]	4.19 ± 0.58	4.62 ± 0.73	0.173
TG [mmol/L]	0.95 ± 0.21	2.22 ± 0.78	< 0.001
HDL-C [mmol/L]	1.37 ± 0.16	1.02 ± 0.21	< 0.001
LDL-C [mmol/L]	2.09 ± 0.50	2.76 ± 0.75	0.001
HOMA-IR	3.85 ± 1.30	7.71 ± 4.38	< 0.001

BMI — body mass index; WC — waist circumference; SBP — systolic blood pressure; DBP — diastolic blood pressure; FBG — fasting blood glucose; TC — total cholesterol; TG — triglycerides; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; HOMA-IR — homeostasis model assessment-insulin resistance index. Data are presented as mean ± standard deviation (SD); $p < 0.05$ means significant difference

($p < 0.01$). In addition, according to the diagnostic criteria for MS, 22 cases of obesity-MS were further diagnosed among the children with obesity. The basic clinical data of children with obesity and children with obesity-MS are analysed in Table 2. The results showed that BMI, WC, SBP, FBG, TG, HDL-C, LDL-C, and HOMA-IR values of children with obesity-MS were all higher than those of children with obesity ($p < 0.01$). In addition, there was no statistical significance in age, sex composition, and DBP between the 2 groups ($p > 0.05$).

Analysis of the expression level and diagnostic value of ZFAS1

The results of qRT-PCR showed that compared with healthy children, the expression level of serum ZFAS1 in children with obesity was up-regulated (Fig. 2A, $p < 0.001$). After further grouping, the expression of ZFAS1 in the obesity-MS group was still significantly higher than that in the simple obesity group (Fig. 2B, $p < 0.001$). The diagnostic value of ZFAS1 in obesity was analysed by ROC method. As shown in Figure 3A, the curve evaluated the diagnostic value of ZFAS1 in healthy children and in all children with obesity. The results showed that the area under the curve (AUC) of the curve was 0.897, and its sensitivity and specificity were 81.7% and 84.4%, respectively, indicating that ZFAS1 has good differential value between healthy children and children with obesity. In Figure 3B, the AUC value, sensitivity, and specificity of the receiver operating characteristic curve (ROC) of ZFAS1 were 0.880, 86.4% and 86.8%, respectively,

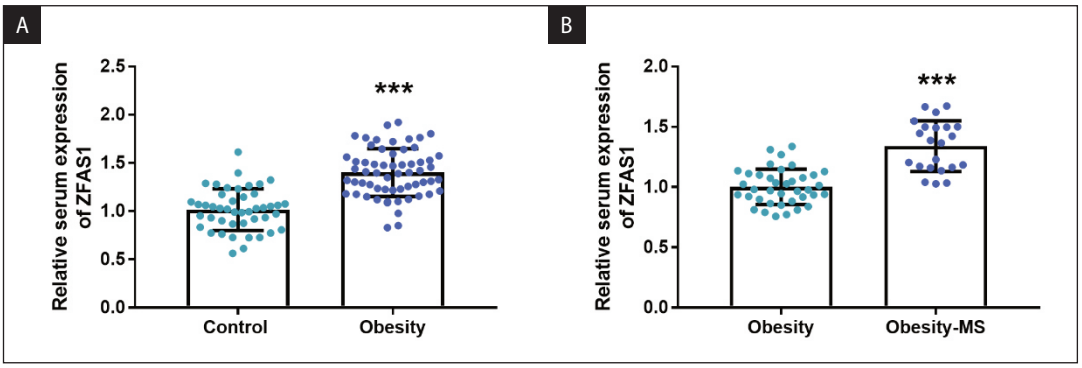

Figure 2. *The expression level of zinc finger antisense 1 (ZFAS1) in serum was detected by quantitative real-time polymerase chain reaction (qRT-PCR).* **A**. *The expression of ZFAS1 in serum of children with obesity was higher than that of healthy children.* **B**. *The expression of ZFAS1 in serum of children with obesity complicated with metabolic syndrome (Obesity-MS) was higher than that of children with obesity (Obesity); $^{***}p < 0.001$*

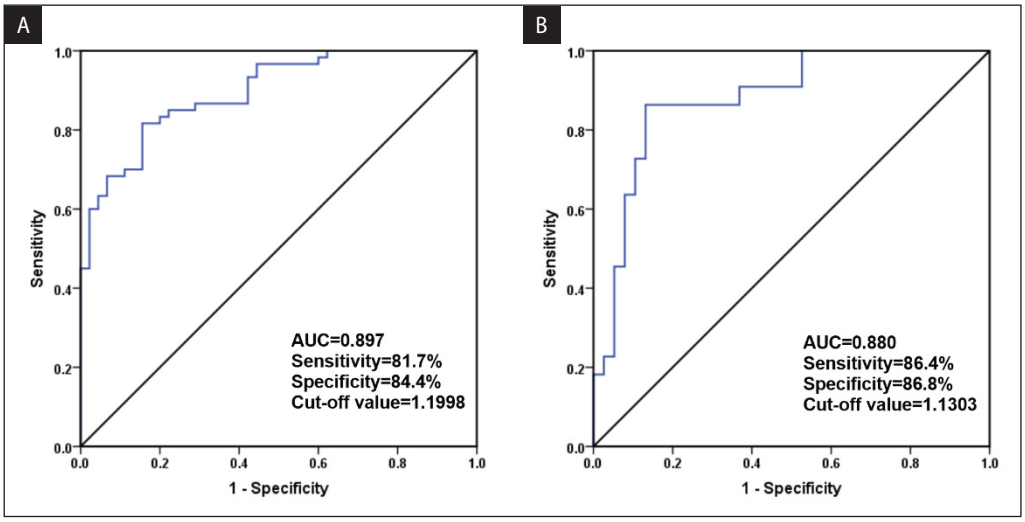

Figure 3. *Receiver operator characteristic (ROC) analysis.* **A**. *The area under the curve (AUC) value of this curve is 0.897, which has high value in distinguishing between children with obesity and healthy children;* **B**. *The AUC value of this curve is 0.880, which can distinguish children with obesity complicated with metabolic syndrome (Obesity-MS) from children with obesity (Obesity)*

Table 3. ***Correlation between zinc finger antisense 1 (ZFAS1) and clinical indicators***

Clinical indicators	r	p
BMI [kg/m^2]	0.622	< 0.001
WC [cm]	0.876	< 0.001
SBP [mm Hg]	0.331	0.010
DBP [mm Hg]	0.697	< 0.001
FBG [mmol/L]	–0.037	0.569
TC [mmol/L]	0.112	0.355
TG [mmol/L]	0.523	< 0.001
HDL-C [mmol/L]	–0.728	< 0.001
LDL-C [mmol/L]	0.671	< 0.001
HOMA-IR	0.598	< 0.001

BMI — body mass index; WC — waist circumference; SBP — systolic blood pressure; DBP — diastolic blood pressure; FBG — fasting blood glucose; TC — total cholesterol; TG — triglycerides; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; HOMA-IR — homeostasis model assessment-insulin resistance index; $p < 0.05$ means significant difference

indicating that ZFAS1 could accurately identify children with obesity-MS among children with obesity.

Association of ZFAS1 with clinical indicators of all children with obesity

The correlation between ZFAS1 and the clinical data of all the children with obesity was analysed by Pearson method, and the results are shown in Table 3. The results revealed that the level of BMI ($r = 0.622$, $p < 0.001$), WC ($r = 0.876$, $p < 0.001$), SBP ($r = 0.331$, $p < 0.05$), DBP ($r = 0.697$, $p < 0.001$), TG ($r = 0.523$, $p < 0.001$), LDL-C ($r = 0.671$, $p < 0.001$), and HOMA-IR ($r = 0.598$, $p < 0.001$) were positively correlated with the expression of ZFAS1, and the level of HDL-C ($r = –0.728$, $p < 0.001$) was negatively correlated with the expression of ZFAS1.

Analysis of possible influencing factors of the transition from obesity to obesity-MS

As shown in Table 4, logistic regression analysis was used to evaluate the clinical data of obesity and obesity-MS children, and the results showed that ZFAS1 (odds ratio [OR] = 6.349, 95% confidence interval [CI] = 1.428-21.034, $p = 0.015$) was an independent influencing factor for the occurrence of obesity-MS in obesity children.

ZFAS1 serves as a miRNA sponge for miR-193a-3p

The complementary sequences and locations of ZFAS1 and miR-193a-3p predicted by StarBase V2.0 are shown in Figure 4A. Furthermore, the data of miR-193a-3p in the serum of children with obesity displayed a reduction trend. It was also found that the expression of miR-193a-3p was further decreased in the serum of children with obesity-MS compared with the obesity group (Fig. 4B, $p < 0.001$). Pearson correlation analysis showed that the expression level of miR-193a-3p in the serum of children with obesity was negatively correlated with the expression of ZFAS1 (Fig. 4C, $r = -0.728$, $p < 0.001$).

Prediction and functional analysis of miR-193a-3p target genes

As shown in Figure 5A and Table 5, three databases, TargetScan, miRDB, and EVmiRNA, were used to predict 104 downstream target genes of miR-193a-3p.

Table 4. *Relationship between different clinical indicators and obesity complicated with metabolic syndrome (Obesity-MS)*

Clinical indicators	OR	95% CI	p
Age [years]	0.793	0.226–2.778	0.717
Sex [male/female]	0.541	0.145–2.022	0.361
BMI [kg/m^2]	3.119	0.824–11.809	0.094
WC [cm]	1.466	0.383–5.605	0.577
SBP [mm Hg]	1.089	0.277–4.273	0.903
DBP [mm Hg]	0.768	0.190–3.109	0.712
FBG [mmol/L]	1.023	0.276–3.787	0.973
TC [mmol/L]	0.880	0.228–3.402	0.853
TG [mmol/L]	3.757	0.904–15.609	0.069
HDL-C [mmol/L]	0.559	0.159–1.961	0.364
LDL-C [mmol/L]	0.678	0.157–2.933	0.604
HOMA-IR	0.581	0.162–2.148	0.415
ZFAS1	6.349	1.428–21.034	0.015

BMI — body mass index; WC — waist circumference; SBP — systolic blood pressure; DBP — diastolic blood pressure; FBG — fasting blood glucose; TC — total cholesterol; TG — triglycerides; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; HOMA-IR — homeostasis model assessment-insulin resistance index; $p < 0.05$ means significant difference

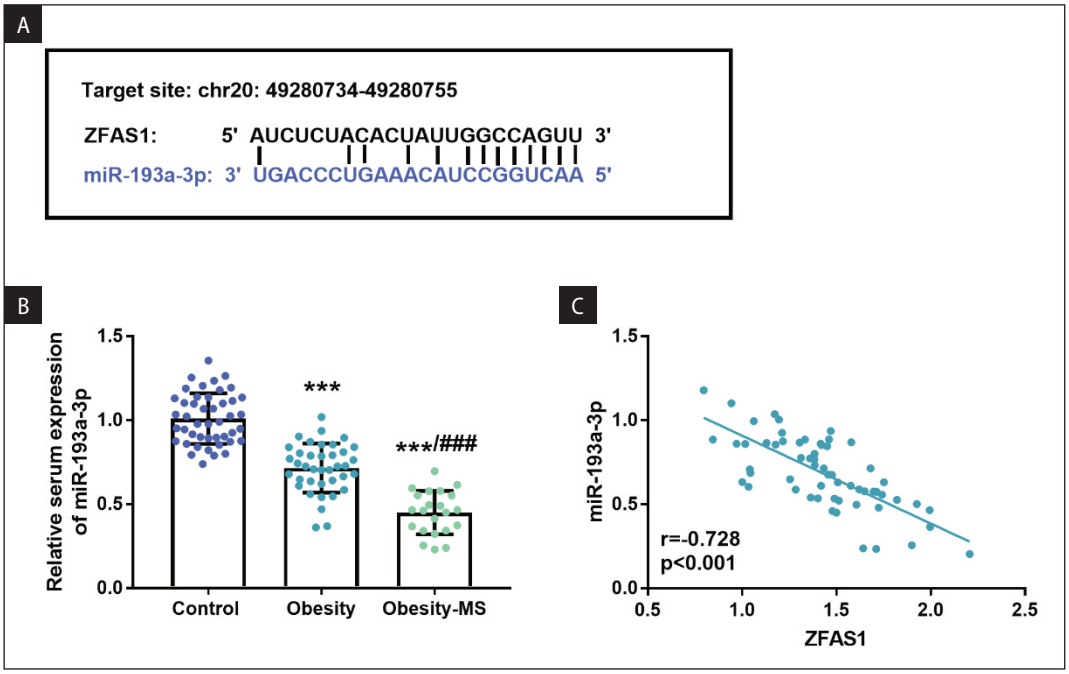

Figure 4. *Verification of the relationship between zinc finger antisense 1 (ZFAS1) and miR-193a-3p.* **A**. *Target binding sites and locations of miR-193a-3p and ZFAS1 predicted by StarBase V2.0 database;* **B**. *The expression of serum miR-193a-3p in children with obesity (Obesity) and obesity complicated with metabolic syndrome (Obesity-MS);* **C**. *Serum expression levels of miR-193a-3p and ZFAS1 in children with Obesity and Obesity-MS; $^{***}p < 0.001$ vs. Control, $^{\#\#\#}p < 0.001$ vs. Obesity*

Figure 5B shows that in Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis, these 104 target genes are mainly enriched in the mitogen-activated protein kinase (MAPK) signalling pathway, Apelin signalling pathway, and oxytocin signalling pathway. In addition, it is also enriched in the related pathways of growth hormone synthesis and gonadotropin secretion.

As shown in Figure 5C, the target genes of miR-193a-3p are mainly located in endocytic vesicles and synaptic vesicles, and they are involved in molecular functions such as protein phosphatase regulation, activation of transcription factor activity, and protein deacetylase binding. In addition, the target genes of miR-193a-3p are involved in a variety of biological processes, includ-

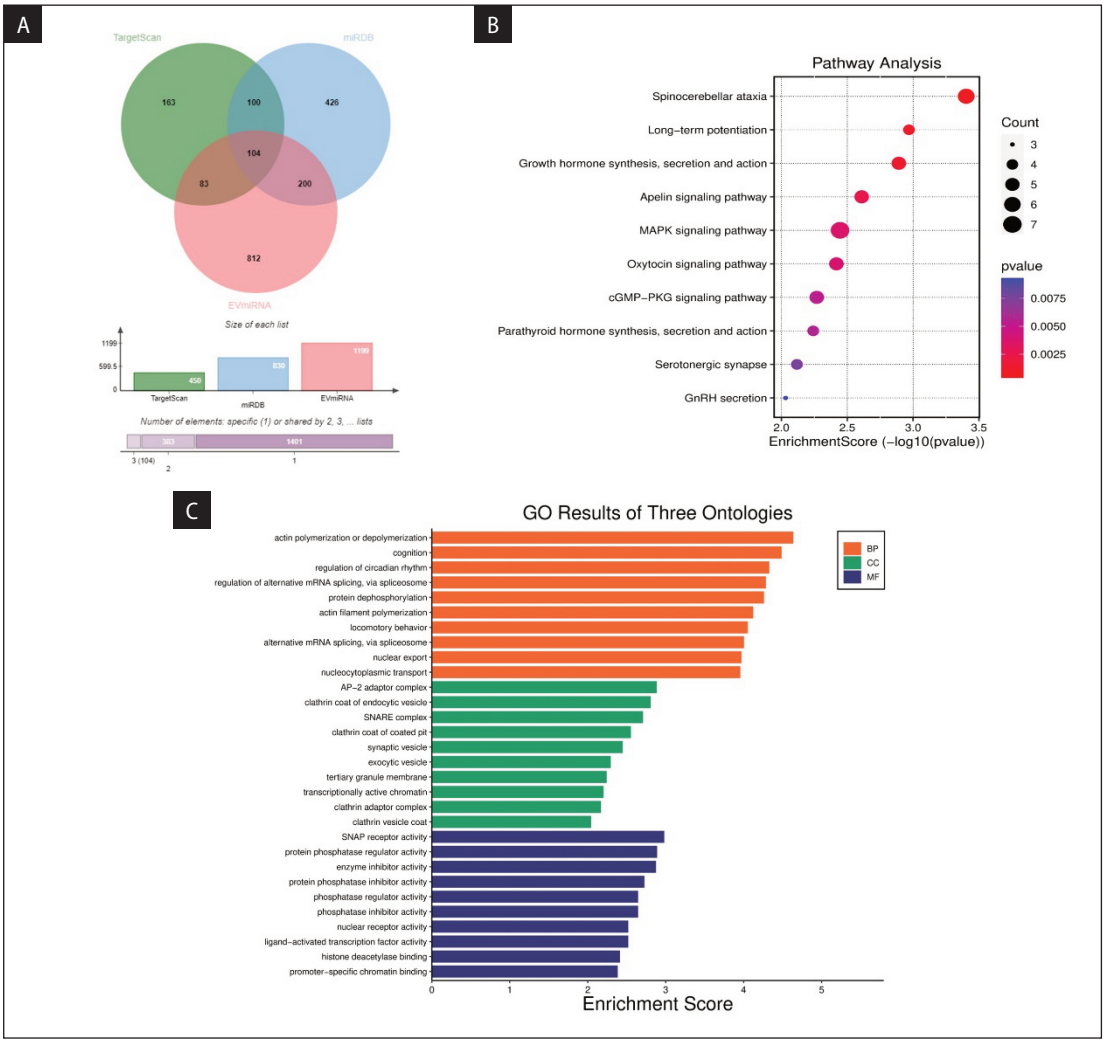

Figure 5. *Bioinformatics analysis of target genes of miR-193a-3p;* **A**. *Venn diagram of downstream target gene prediction results of miR-193a-3p by TargetScan, miRDB and The Extracellular Vesicles miRNA Database (EVmiRNA);* **B.** *Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis of downstream target genes of miR-193a-3p.* **C**. *Gene ontology (GO) analysis of downstream target genes of miR-193a-3p*

ing actin polymerisation and depolymerisation, protein dephosphorylation, mRNA splicing, and nucleocytoplasmic transport.

Discussion

The expression of serum ZFAS1 and miR-193a-3p increased and decreased in children with simple obesity, respectively. Besides, ZFAS1 and miR-193a-3p were found to be further up-regulated and down-regulated in children with obesity-MS, emphasising the important role of ZFAS1 and miR-193a-3p in obesity and obesity-MS. The differential expression of serum ZFAS1 showed high diagnostic accuracy in distinguishing children with simple obesity from healthy children, and those with obesity-MS from those with simple obesity. Among the included indicators, BMI, SBP, DBP, and others had significant positive or negative correlation with ZFAS1, and the elevation of ZFAS1 was determined as an independent risk factor for the development of simple obesity into obesity-MS.

Obesity is a complicated chronic disease, which is caused by many factors such as heredity, environment, and behaviour [17]. With the improvement of living standards and the change of diet structure, obesity is increasingly prevalent among people of all ages. At present, various methods for treating obesity are emerging in an endless stream, and the single treatment mode aimed at weight loss is not effective, mostly because the individual differences and metabolic characteristics of patients are ignored [18]. It is almost difficult to see the difference between patients with obesity and with obesity-MS from their appearance, but the latter is often accompanied by metabolic abnormalities such as diabetes, hypertension, hyperlipidaemia, and insulin resistance. Clinically, there is no

Table 5. *The target genes of miR-193a-3p*

SLC25A3	GRM8	CUL4B	MSL2	MGAT5	ASAP1	UHMK1
NMB	VAMP7	TXNRD1	MTOR	ENAH	CACNA1C	ZNF652
AKR1D1	ELMOD1	SRSF7	SNAP25	ELAVL2	GHR	TOMM20
PRCC	NDUFC1	TTC37	QKI	STRN	XIAP	RORA
PPP1CC	JARID2	PPP4R2	NAA50	UBE2O	PTPRB	FAM172A
PACRGL	GAN	DLX3	NCKAP1	MAGED1	LZIC	ATXN1
FAM214A	ZC3H12C	ARHGEF9	GOSR1	DCUN1D1	GNAQ	TUBB
CASP3	MTA1	ARX	SGIP1	MXI1	RUNX1T1	GAS7
ARPP19	SPR	CRIM1	MEIS2	MEF2C	NCAM1	CHD2
NDFIP1	AFF4	FAM122A	SUPT16H	THRB	EPS8	NIPBL
NRAS	DNAJC27	GSPT1	SP3	ELK4	FAM84B	CLCN3
RASA1	FGF14	PDE4B	ANKRD17	SYT14	PICALM	NFYA
CTDSPL2	RDX	IPO5	NR3C2	EIF2S2	DUSP16	HNRNPA1
DDX17	FAR1	MSI2	ATAD2B	PRUNE2	ZNF609	PIGA
CAPZA1	LPGAT1	TBC1D9	KCNJ10	CHD7	MEF2D	

reliable and effective marker to distinguish obesity-MS from simple obesity. Although BMI is often used as an important reference in the diagnosis and treatment of obesity, studies showed that it cannot correctly identify and predict obesity-related complications [19]. In the current study, ZFAS1 was found to be dysregulated in obesity and obesity-related complications, such as MS. The data show that ZFAS1 has the lowest expression level in the serum of healthy people, followed by children with simple obesity, and the highest expression level in obesity-MS. This stepped expression trend may be more helpful for ZFAS1 to play its clinical diagnostic role. Here, we divided all subjects into 2 study groups according to the expression trend of ZFAS1. One group comprised healthy children and children with obesity, and the other group was children with obesity and children with obesity-MS. ROC analysis demonstrated that ZFAS1 can distinguish obesity-MS from obesity at different cutoff values, suggesting that ZFAS1 may be a valuable biomarker candidate to distinguish obesity-MS from obesity. Although a lot of research has linked ZFAS1 to cancer, it has also been found to be associated with fat accumulation in some cancers. For example, downregulating the expression of ZFAS1 plays a role in inhibiting lipogenesis in colorectal cancer, thereby reducing fat accumulation [20]. In a study of pancreatic cancer, ZFAS1 gene knockout significantly inhibited the proliferation of cancer cells, while reducing the content of free fatty acids, TC, and TG [21]. It is known that obesity is one of the risk factors for cardiovascular and cerebrovascular disease. In an atherosclerosis-related study, ZFAS1 was found to be increased in a high-fat fed mouse model [22]. This evidence suggests that ZFAS1 may be involved in fat formation and accumulation, but more research is needed to explore this speculation.

In recent years, more and more studies have found that the dysregulation of miRNA is involved in the occurrence of obesity and related complications. MiR-143 specifically promotes adipocyte differentiation by down-regulating extracellular signal-regulated kinase 5 [23]. Elevated miR-802 in the liver of patients with nonalcoholic fatty liver disease inhibits AMPK activity and accelerates steatosis and inflammation [24]. As a tumour suppressor, miR-193a-3p has been found to be abnormally expressed in some tumours and inflammatory diseases in the past, such as colorectal cancer and endometritis [25, 26]. Meerson et al. detected decreased expression of miR-193a-3p in the abdominal subcutaneous adipose tissue samples of non-diabetic subjects, and the expression level of this gene was negatively correlated with BMI, suggesting that miR-193a-3p may play a role in fat formation and metabolism [27]. StarBase V2.0 predicted that miR-193a-3p was a direct target of ZFAS1, and the targeting relationship was verified earlier in a study on hepatoblastoma by Cui et al. [28]. In this study, the expression of miR-193a-3p in the serum of healthy children, children with obesity, and children with obesity-MS showed a progressive downward trend, which was opposite to the expression trend of ZFAS1. Further analysis showed that the expression of miR-193a-3p in the serum of obesity subjects was negatively correlated with the expression of ZFAS1. This result reflects the targeting relationship between miR-193a-3p and ZFAS1.

Previous studies have found that miR-193a-3p, as a tumour suppressor, is down-regulated in tumour tissues, thus losing control over the growth and migration of tumour cells, leading to the occurrence of various cancers [29]. Subsequent studies have shown that miR-193a-3p plays a role in human inflammation. In this study, miR-193a-3p expression was reduced in obese patients. The regulation of downstream targets of miR-193a-3p to promote obesity development remains largely unknown. We used bioinformatics technology to identify 104 downstream target genes of miR-193a-3p. Through GO analysis and KEGG analysis, it was found that these target genes are enriched in many biological processes such as metabolic processes, hormone synthesis and secretion, and protein phosphokinase regulation. In addition, these genes are mainly enriched in MAPK, apelin, and oxytocin signalling pathway, which play important roles in the development of obesity [30–32]. The association between obesity and Apelin signalling pathway has been observed in many studies, showing that Apelin, as an adipokine, plays an important role in body fluid balance, angiogenesis, energy metabolism, and other processes [33]. The oxytocin signalling pathway has important implications in the treatment of obesity by inducing fat loss in rats on a high-fat diet through increased satiety response and lipid utilisation [34]. In summary, through bioinformatics analysis, we have preliminarily learned the possible molecular functions and enrichment pathways of the downstream target genes of miR-193a-3p, and the regulation of these functions and pathways is significantly related to obesity.

The limitations of this study are as follows. First, this study is a single-centre study with a small sample size, which may have selection bias. Second, this study did not explore the potential mechanism by which ZFAS1 promotes obesity. Based on the above limitations, in future, larger longitudinal studies are needed to validate gene expression trends in large multicentre samples to ensure more reliable results. Besides, future studies should focus on the mechanism of ZFAS1 promoting obesity. This requires us to explore the signalling pathways and molecular interactions involved in ZFAS1 *in vitro* or *in vivo* studies. At the same time, exploring the therapeutic effect of ZFAS1 on obesity and the improvement effect on metabolic indexes in animal studies may help to better clarify ZFAS1 as a diagnostic marker and therapeutic target for obesity.

Conclusions

In conclusion, this study revealed that ZFAS1 was highly expressed in children with simple obesity and obesity-MS. ZFAS1 has shown advantages in distinguishing obesity-MS from obesity, and its level was significantly correlated with an abnormal increase of clinical indicators in all children with obesity. ZFAS1 is a risk factor for the progression of obesity to obesity-MS. This study provided a basis for finding diagnostic biomarkers of obesity-MS, and it supplied a theoretical basis for further exploring the molecular mechanism of ZFAS1 in obesity.

Data availability statement

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

Ethics statement

This study was carried out after obtaining approval from the Ethics Committee of Taihe Hospital, Affiliated Hospital of Hubei University of Medicine.

Authors' contributions

X.J.L., C.X., and D.D.T. designed the research study. X.J.L., C.X., X.J.T., and H.Z.Z. performed the research. X.J.L., C.X., and D.D.T. analysed the data. X.J.L. and C.X. wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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