RELATIONSHIP BETWEEN FRA-2 PROTEIN, MRNA EXPRESSION, DNA METHYLATION LEVEL AND THE OCCURRENCE OF METABOLIC SYNDROME IN XINJIANG UIGHUR POPULATION

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ABSTRACT

Objective: *Fos-related antigen-2 (FRA-2) plays an important role in metabolism. However, few people have studied whether the expression of FRA-2 will be disordered when metabolic syndrome (MetS) and other risk factors appear. In our research, the expression of FRA-2 protein, mRNA and DNA methylation in peripheral blood of Xinjiang Uygur non-metabolic population and metabolic syndrome population was detected, and the relationship between FRA-2 DNA methylation and MetS composition was further analyzed.*

Methods: *100 Uygur subjects were divided into non-metabolic syndrome (Non-MetS, n=50) and metabolic syndrome (MetS, n=50) groups. The general data and biochemical indexes were recorded. The FRA-2 protein was detected by ELISA and mRNA was detected by RT-PCR. The DNA methylation level of FRA-2 was analyzed by MassARRAY spectroscopy.*

Results: *Compared with Non-MetS group, (1) the waist circumference, FPG, HbA1c, TG, TC, LDL-C,SBP, DBP, BMI, FINS and HOMA-IR in MetS group increased, while HDL-C decreased. (2) The expression of FRA-2 protein and mRNA in the MetS group was down-regulated; the methylation of 8 CPG units in the MetS group increased. (3) The average methylation rate of FRA-2 was negatively correlated with mRNA expression. (4) 1 CPG unit of FRA-2 was positively correlated with SBP, 3 CPG units were positively associated with DBP, 5 CPG units were positively associated with waist circumference, 6 CPG units were positively associated with FPG, TG, BMI and HOMA-IR.*

Conclusion: *The down-regulation of FRA-2 protein and mRNA expression and the up-regulation of DNA methylation may have correlation with the occurrence and development of MetS in Xinjiang Uygur.*

Keywords: *Metabolic syndrome, FRA-2, DNA methylation, uighur, relationship.*

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Introduction

Metabolic syndrome (MetS) is a type of metabolic disorder which is combined with multiple cardiovascular risk factors such as hyperglycemia, overweight or abdominal obesity, hypertension, and lipid metabolism disorders. Abdominal obesity and insulin resistance are the significance pathophysiological mechanisms of MetS[(1)]. With the improvement of the living standards in recent years, the prevalence rate of MetS is also showing a upward trend. Based on the statistics, the prevalence rate of metabolic syndrome is 24.2% in China. The complications of MetS include cardiovascular disease[(2)], malignant tumor[(3-4)], which have high incidence rate and mortality rate and bring great financial burden to families and society as well as become a global health problem. Xinjiang is a multi-ethnic region, and the prevalence rate of MetS exceeds the national average level, especially

the Uygur population, with a prevalence rate of 26.50%[5-6]. FOS-related antigen-2 (FRA-2) is a member of the transcription factor activating protein 1 (AP-1) family. FRA-2 is found in various tissues and cells of animals and human beings, and is related to cell proliferation, apoptosis and differentiation. Abnormal FRA-2 expression may induce pathological changes in vivo, and participate in the occurrence of human diseases. FRA-2 is a significant regulator of leptin gene expression in adipose cell, and is involved in regulating the energy balance, carbohydrate and lipid metabolism and insulin resistance in mammals. Leptin is closely related to the occurrence of metabolic diseases including MetS[8-10].

Related studies have found[11] that DNA methylation is related to mammalian development and the occurrence of a variety of diseases, and methyl is added to CPG sequences in the promoter region. The expression of DNA methylation is influenced by age, environment and lifestyle, and may be related to MetS[12].

However, the pathogenic mechanism of FRA-2 in the occurrence of MetS in Xinjiang Uygur population remains unclear. Therefore, exploring the expression of FRA-2 protein, mRNA and DNA methylation in Xinjiang Uygur population of MetS contributes to reveal the pathogenesis of MetS in Uygur population and provide a basis for targeted treatment of MetS.

Materials and methods

Objects of Research

In the aggregate, 100 subjects were enrolled in XinJiang, from January 2020 to October 2020. Due to the special diet of Xinjiang Uyghurs, we adopt the Chinese definition of MetS, according to the definition of MetS by Chinese Diabetes Society(CDS 2017), subjects were classified into Non-MetS group (n=50) and MetS group (n=50).

CDS 2017:

• Central obesity: Waist circumference >80 cm (women) or >90 cm (men);

• Hyperglycemia: FPG≥6.1mmol/L or 2hFBG ≥7.8mmol/L or diagnosis of diabetes;

• Hypertension: Medication for hypertension or blood pressure >130/85 mmHg;

• High triglyceremia: TG ≥1.7mmol/L or treat elevated triglycerides;

• HDL<1.04 mmol/L.

Exclusion criteria included:

• Patients with various severe liver, kidney, blood system diseases, cardiovascular and cerebrovascular system diseases and tumors;

• Patients with various acute and chronic inflammatory diseases or obvious infection in recent 2 weeks;

• Patients with autoimmune diseases, long-term use of immunosuppressants or other endocrine diseases;

• Patients with surgery or recent trauma;

• Patients with history of drug abuse;

• Patients with pregnancy and lactation;

• Patients engaged in toxic and harmful works.

Data Acquisition

The data of the subjects was shown in Table 1. Body mass index (BMI) = body mass (kg)/height $(m)^2$, Systolic and diastolic blood pressure of subjects were measured with mercury sphygmomanometer when they had 15 minutes' rest.

The measurements were made every 2-5 minutes and repeated for 3 times, the average of all three times was calculated. Fasting blood glucose (FPG), high density lipoprotein cholesterol (HDL-C), triacylglycerol (TG) and other biochemical indicators were measured by automatic biochemical analyzer (BS-280, Mindray, China). Glycosylated hemoglobin (HbA1c) was detected by high performance liquid chromatography (AC6601, Adikang Medical Technology Co., Ltd, China). Automatic electrochemiluminescence immunoassay analyzer was used to analyze fasting insulin. Homeostasis model assessment was used to calculate HOMA-IR (HOMA-IR=FINS×FPG/22.5). Our protocol was approved by the Ethics Committee of the First Affiliated Hospital of Shihezi University School of Medicine, and all subjects signed the informed consent form.

Determination of FRA-2 Protein Expression by ELISA

The expression of FRA-2 protein was determined using FRA-2 enzyme-linked immunospecific assay kit (R&D Systems, MN, USA).

Extraction of total RNA from Leukocytes

Total RNA was extracted by total RNA Kit (Solaibao biochemical reagent supplier, Beijing, China). DNA from peripheral blood was extracted with DNeasy Blood and Tissue Kit (Qiagen, Germany).

The concentration and purity of DNA were detected by spectrophotometer, the absorption of nucleic acid at 260 nm was quantified.

Determination of FRA-2 mRNA expression by RT-PCR

The cDNA template was synthesized according to the instructions of cDNA first strand synthesis kit (APExBIO, USA) and detected with RT-PCR. The primer sequence of FRA-2 was[13]: forward 5'-CCAGATGAATGTCATGGC-3', reverse 5'-CTCCGGTTTGGTAGATTGGA-3', primers of β-actin was forward 5'-CCCAGCACAATGAAGATCAAGATCAT-3' and reverse 5'-ATCTGCTGGGTGGACAGCG-3'.

Reaction conditions of RT-PCR involved 94 °C for 4 min, 72 °C for 2 min followed by 35 cycles of 94 °C for 20 s, 56 °C for 30 s and 72°C for 60s.

The mRNA level of FRA-2 was analyzed using were analyzed by RT-PCR using GelDoc EZ automatic gel imaging analysis system (UVB company, USA).

Methylation of FRA-2 gene

DNA was modified by sodium bisulfite in the following reaction conditions: 95 °C for 30s, 50 °C for 15 min followed by 20 cycles.

The primer sequence of FRA-2 was[13]:forward 5'-AGGAAGAGAGGTAGGTTTAGGAGAGGGGTGTG-3' and reverse 5'-CAGTAATACGACTCACTATAGGGAGAAGGCTACAACCCCCAAAACTTAACTAAAAC-3'. The CPG primer of FRA-2 was employed to amplify DNA by sulfite.

The reaction conditions of PCR involved 94°C preheating for 4 min, 72 °C annealing for 3 min, then 45 cycles of 94 °C for 20 s ,56 °C for 30 s and 72°C for 1min. PCR products were using 384-pad spectral chip (JL-PZY96BT, Shanghai, China).

Mass spectra were gathered through employing MassARRAY mass spectrometer (Bruker-Sequenom), EPITYPER software generates methylation data.

Statistical analysis

The data were processed by SPSS 26.0. Data with normal distribution were tested by the Student t-test and presented as $\bar{x}\pm s$.

Data with non-normal were analyzed by Mann-Whitney U-test and presented as M (P25, P75). The relationship of the indexes by employing Spearman method. $P<0.05$ was considered statistically significance.

Results

Comparison of clinical and metabolic indexes between groups

Compared with Non-MetS group, waist circumference, BMI, SBP, DBP, FPG, TC, TG, LDL-C, HbA1c, FINS and HOMA-IR in MetS group increased, while HDL-C decreased. (Table 1).

Parameter	Non-MetS group	MetS group
Cases	50	50
Gender (female/male)	26/24	25/25
Age (years)	52.12±13.11	54.36±8.50
WC	90.42±10.26	104.24±9.62**
BMI (kg/m^2)	24.24±2.94	29.05±3.74**
SBP (mmHg)	129.40±20.44	138.16±19.13**
DBP (mmHg)	81.60±15.59	89.98±14.87**
HbA1c (%)	6.70±1.87	9.29±2.44**
FPG (mmol/L)	6.31±2.51	10.25±4.80
LDL-C (mmol/L)	2.23±0.67	2.60±0.69*
HDL-C (mmol/L)	1.17 (0.98, 1.49)	1.05 (0.97, 1.24)*
Triglyceride (mmol/L)	1.22 (0.95, 1.31)	2.05 (1.59, 2.55)**
Total cholesterol (mmol/L)	3.92±0.89	4.94±1.03**
FINS (mIU/L)	9.51±1.33	13.20±1.01*
HOMA-IR	2.79 (1.63, 3.81)	5.90 (3.29, 9.46)**

Table 1: Comparison of clinical and metabolic indexes between groups [$\bar{x}\pm s$ or M (P_{25}, P_{75})].
*WC (Waist circumference); BMI (body mass index); SBP (systolic blood pressure); DBP (diastolic blood pressure); HbA1c (hemoglobin Alc); FPG (fasting plasma glucose); LDL-C (low density lipoprotein cholesterol); HDL-C (high density lipoprotein cholesterol); FINS (fasting plasma insulin); HOMA-IR (index of insulin resistance); P-value: *<0.05, **<0.01.*

Difference expresion of FRA-2 protein and mRNA levels between groups

Compared with Non-MetS group, the expression of FRA-2 protein in MetS group was down-regulated, 32.04 (15.37, 53.59) VS 11.72 (6.50, 24.18), and FRA-2 mRNA in MetS group was down-regulated, 2.11 (1.95, 2.97) VS 1.05(0.96, 1.21). (Figure 1, Figure 2).

Comparison of methylation levels of FRA-2 between groups

Compared with Non-MetS group, the methylation levels of FRA-2 in CPG units (CPG 1, CPG 3, CPG 4.5, CPG 8, CPG 9.10, CPG 12.13.14, CPG 15.16.17, and CPG 19) increased in MetS group. (Figure 3, Figure 4).

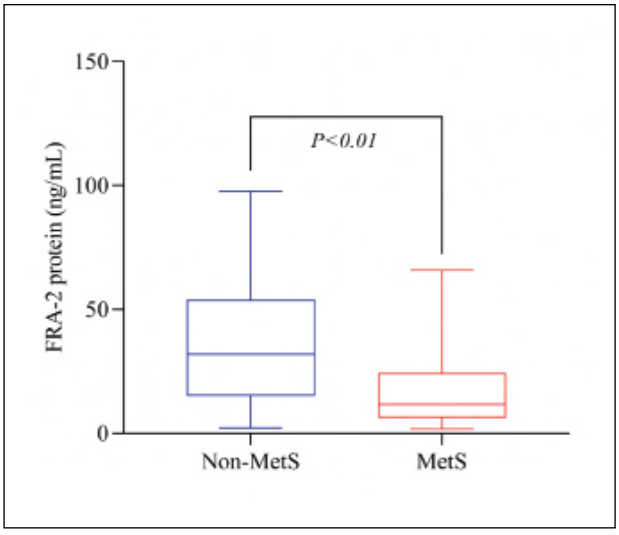

Figure 1: Difference expresion of FRA-2 protein levels between groups.

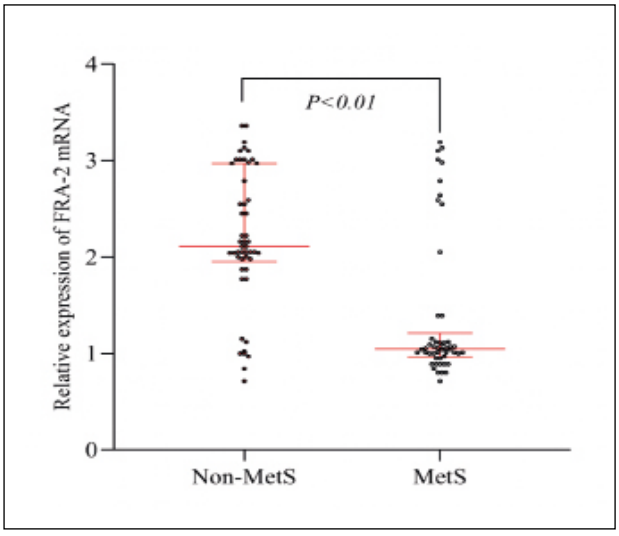

Figure 2: Difference expresion of FRA-2 mRNA levels between groups.

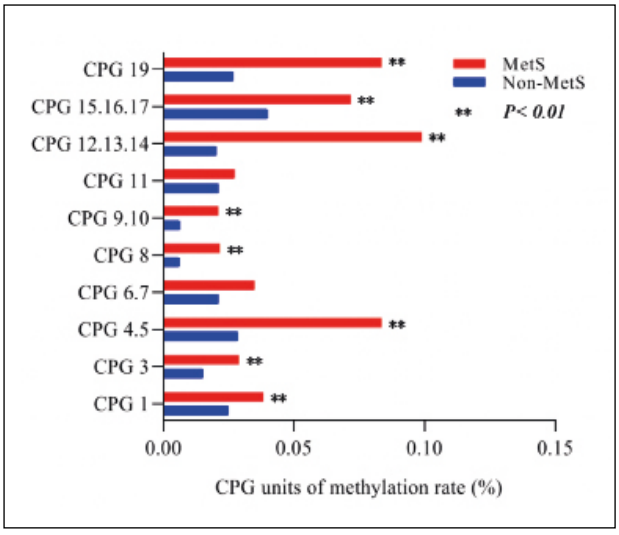

Figure 3: Comparison of methylation levels of FRA-2 between groups.

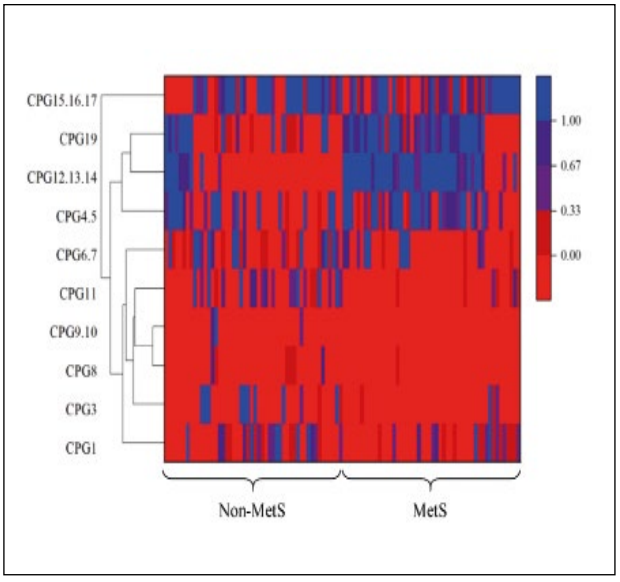

Figure 4: Hierarchical clustering of FRA-2 methylation between groups (Showing 1 topic per column, each line displays 1 CPG unit. The color coding reflects methylation level, red is 0 percent and blue is 100 percent).

Difference of mean methylation levels of FRA-2 between groups

Compared with Non-MetS group, the mean methylation levels of FRA-2 in the MetS group was increased, 0.02(0.01, 0.03) VS 0.05 (0.04, 0.06). (Figure 5).

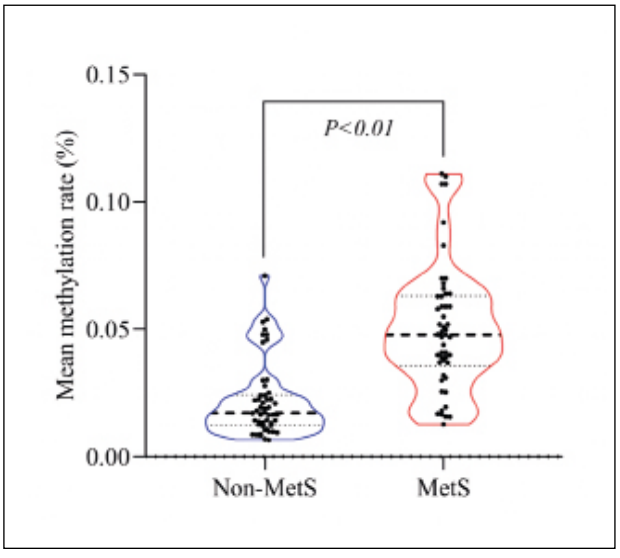

Figure 5: Comparison of mean methylation levels of FRA-2 between groups.

Correlation analysis between average methylation levels of FRA-2 and mRNA expression

Spearman correlation analysis revealed average methylation level of FRA-2 was negatively correlated with mRNA expression. (Figure 6).

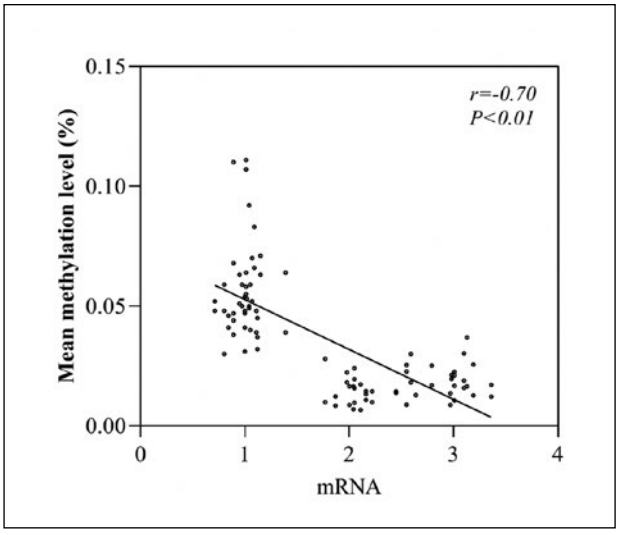

Figure 6: Correlation between methylation level of FRA2 gene and mRNA expression.

Relationship analysis between methylation of CpG units and composition of MetS

Spearman correlation analysis revealed that methylation of CPG 4.5, CPG 8, CPG 9.10, CpG 12.13.14 and CPG 19 were positively correlated with WC; CPG 3, CPG 4.5, CPG 8, CPG 9.10, CPG 12.13.14 and CpG 19 were positively correlated with BMI, FPG and HOMA-IR; CPG 8 and CPG 19 were positively correlated with DBP; CpG 4.5, CPG 8, CPG 9.10, CPG 12.13.14, CPG 15.16.17 and CPG 19 were positively correlated with TG. (Table 2).

CPG units	CPG 1	CPG 3	CPG 4.5	CPG 8	CPG 9.10	CPG 12.13.14	CPG 15.16.17	CPG 19
WC	0.064	0.194	0.371**	0.347**	0.436**	0.566**	0.057	0.446**
BMI	-0.007	0.238*	0.283*	0.238*	0.311**	0.442**	0.058	0.294**
SBP	0.035	-0.073	0.006	0.203*	0.095	0.096	0.118	0.137
DBP	0.096	-0.056	0.104	0.230*	0.081	0.134	0.202*	0.206*
FPG	0.078	0.472**	0.676**	0.612**	0.611**	0.745**	0.051	0.705**
TG	0.077	0.204*	0.353**	0.332**	0.396**	0.496**	0.192	0.403**
HDL-C	-0.072	-0.022	-0.095	-0.037	-0.055	-0.085	-0.058	-0.049
HOMA-IR	-0.032	0.374**	0.480**	0.405**	0.495**	0.552**	-0.021	0.584**

Table 2: Relationship analysis between methylation of CPG units and composition of MetS.
*P-value: *<0.05, **<0.01.*

Discussion

The incidence of MetS is gradually increasing, and its complications have also brought a huge burden to patients and society. Therefore, a sustainable solution to MetS is urgent. Due to the high-salt and high-fat diet, the incidence of MetS of Xinjiang Uygur population is higher than the national average. Previous studies[15] have analyzed the pathogenesis of MetS, including diet structure, inflammatory factors and oxidative stress, but there have few researches on the methylation of FRA-2 protein, mRNA and DNA in the pathogenesis of MetS in Xinjiang Uygur population from the molecular mechanism point of view. We aimed to analyze the expression of FRA-2 protein, mRNA and DNA methylation in the Xinjiang Uyghur MetS population, and to explore the effect of FRA-2 DNA methylation in the occurrence of MetS in Uyghur population.

Our study found that the expression of FRA-2 protein and mRNA in MetS group were significantly lower than that in Non-MetS group, which indicated that the decrease of FRA-2 protein and mRNA expression may lead to MetS in Xinjiang Uygur population. Nunes-Souza[16] et al. confirmed the relationship between DNA methylation and MetS in other genes. Therefore, we speculated that the similar relationship may also exist in FRA-2. Then, we analyzed the methylation of the CPG site of FRA-2. The methylation level of 7 CPG units in MetS group was higher than Non-MetS group, and the mRNA expression of FRA-2 decreased with the increase of the average methylation level of DNA. These results indicated that the mRNA expression of FRA-2 gene was down-regulated by hypermethylation, and thus participating in the occurrence and progress of MetS.

Our study discovered that BMI, HOMA-IR and WC in MetS group were higher than Non-MetS group, which was consistent with other related studies[17] and further indicated that the Xinjiang Uygur population had central obesity and insulin resistance. Samblas M[18] et al. found that DNA methylation was involved in obesity and induced insulin resistance in MetS patients, and FRA-2 gene may be involved in it. Related studies found that methylation of multiple CPG sites of FRA-2 gene was positively correlated with BMI, IR and waist circumference, which indicated that hypermethylation of FRA-2 gene DNA was the influencing factor of central obesity and insulin resistance in MetS patients of Xinjiang Uygur population. Abi-Ayad M[19] et al. found that MetS patients often showed the characteristics of glucose and lipid metabolism disorder.

Compared with non-MetS group, FPG and TG were increased and HDL-C was decreased in MetS patients, which was consistent with our study. Studies reported that[13] the disorder of glucose and lipid metabolism were affected by DNA methylation of

FRA-2 gene. We found that the CPG site methylation of FRA-2 gene was positively correlated with FPG and TG, suggesting that the DNA hypermethylation of FRA-2 gene was the influencing factor of FPG and TG in Xinjiang Uygur Mets population. However, related studies found that there was no relationship between the increase of methylation level of FRA-2 gene and the decrease of high density lipoprotein cholesterol level, which may be due to the small number of subjects with low HDL-C or the different genetic background in Xinjiang.

Elevated blood pressure is crucial to diagnose MetS. Compared to Non-MetS group, SBP and DBP were higher in MetS group, which was consistent with Clifton et al.[20]. Some scholars[21] believe that DNA methylation paticipate in the occurrence of cardiovascular diseases such as hypertension. Our study found that the methylation of CPG site of FRA-2 gene was positively correlated with diastolic blood and systolic blood pressure, which indicated that the increased methylation of FRA-2 gene was an influencing factor of elevated blood pressure in Xinjiang Uygur MS patients. In recent years, some studies[22-24] reported that FRA-2 might regulate leptin expression, induce sympathetic nerve activation, and affect leptin-aldosterone pathway, which was related to obesity-related hypertension in mammals and require to be further proved by expanding the sample size in the future. The object of this study only included the Uyghur population, and the sample size was limited. It is necessary to perform more studies on MetS population of different regions and nationalities and its molecular mechanism in the future, and FRA-2 may become a potential target for research on the pathogenesis of MetS.

In conclusion, metabolic disorders such as central obesity, hyperglycemia, hyperlipidemia, hypertension and insulin resistance in MetS population has the ability to down-regulate the expression of mRNA and protein through hypermethylation of FRA-2 gene DNA, and may be involved in the occurrence and development of MetS. FRA-2 methylation status may be a new biomarker for the MetS in Xinjiang Uygur population, and become a potential new target for drug treatment.

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Data Availability:
The data have not been placed in any online data storage. The datasets generated and analyzed during the study are available upon request from the Corresponding author.

Authors' Contributions:
Jun Li, ZeXin Hou, and Siyuan Li share the first author.

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