

BMJ Open Influence of lifestyle on the *FAIM2* promoter methylation between obese and lean children: a cohort study

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ABSTRACT

Objective: An obesity-related gene, Fas apoptotic inhibitory molecule 2 (*FAIM2*), is regulated by nutritional state and the methylation levels of the *FAIM2* promoter are significantly associated with obesity. Lifestyle factors, such as sedentary behaviour and physical activity, might modify epigenetic patterns that have been related to obesity. Whether the molecular mechanisms by which *FAIM2* affects obesity are involved in lifestyle is unclear. This study investigates the potential differences of the *FAIM2* promoter methylation with sedentary behaviour and physical activity in obese and lean children.

Design: Cohort study.

Setting: Institute of Pediatrics in China.

Participants: 59 obese cases and 39 lean controls aged 8–18 years recruited from a cross-sectional survey of children from Beijing in 2013.

Primary and secondary outcome measures: The *FAIM2* promoter methylation was quantified using the Sequenom MassARRAY platform. Sedentary behaviour and physical activity were investigated using a questionnaire. The influences of different lifestyles on methylation variations in obese and lean children were examined by multiple linear regression.

Results: The methylation levels at seven CpG sites of the *FAIM2* promoter were significantly associated with sedentary behaviour, especially the methylation levels at site –975, site –413, sites –362 and –360, and sites –353 and –349 ($p=0.00004$, 0.00009 , 0.0006 and 0.00005 , respectively). There were significant differences between the methylation levels at four CpG sites in obese and lean participants with high or moderate physical activity level <150 min/week.

Conclusions: This study provides the first evidence that there are significant differences in the associations of the *FAIM2* promoter methylation with sedentary behaviour and physical activity between obese and lean children. Our results suggest that lifestyle may possibly be mediating the process of the *FAIM2* involved in obesity.

INTRODUCTION

In recent years, the rising incidence of obesity has been a major threat to public health worldwide.¹ Obesity most likely results

Strengths and limitations of this study

- This is the first study to evaluate the methylation levels of the Fas apoptotic inhibitory molecule 2 (*FAIM2*) promoter and lifestyle factors.
- This study may not provide direct evidence that the expression of *FAIM2* influences obesity.
- This study investigated the methylation levels in peripheral blood leucocytes, but not in the hypothalamus or adipocytes.

from the complex interaction between genes and lifestyles such as physical activity, diet and behaviour.² Current evidence indicated that lifestyle factors might modify epigenetic patterns,³ and DNA methylation has been suggested to be associated with the development of obesity.⁴

Fas apoptotic inhibitory molecule 2 (*FAIM2*), a 35.1 kDa membrane protein highly expressed in the hippocampus, regulates Fas ligand-mediated apoptosis in neurons.⁵ The single nucleotide polymorphism (SNP) rs7138803 near *FAIM2* was reported to be associated with obesity based on genome-wide association (GWA) studies in Caucasians,⁶ and replicated studies showed that this SNP was significantly associated with obesity in Asian populations.^{7 8} *FAIM2* was regulated by nutritional state⁹ and the methylation levels of the *FAIM2* promoter were significantly associated with obesity,¹⁰ but whether the molecular mechanism by which *FAIM2* affects obesity is involved in lifestyle has not been clarified.

Because obesity-related lifestyle factors might modify epigenetic patterns and the methylation levels of the *FAIM2* promoter are significantly associated with obesity, we hypothesise that lifestyle factors play a role in the methylation of the *FAIM2* promoter. The aim of this study was to investigate the differences of the methylation levels of the *FAIM2* promoter between obese and lean participants according to different sedentary behaviour and physical activity.

METHODS

Population

The cohort included 59 obese and 39 lean participants randomly recruited from a cross-sectional survey of children from Beijing aged 8–18 years in 2013. The survey was a physical fitness and health surveillance of Beijing school students, and included a questionnaire, medical examination, anthropometric measurement and collected venipuncture blood samples ($n=3143$; boys 50%). Obesity was diagnosed by the Chinese age-specific and sex-specific body mass index (BMI) cut-offs (see online supplementary table S1) and fat mass percentage (FMP) >40 . Leanness was diagnosed by WHO BMI cut-offs and FMP <15 . The research budget limited the sample size in the study. In future studies, we hope to examine the methylation levels of the *FAIM2* promoter in greater sample sizes.

We obtained written informed consent from all participants (or their parents/guardians).

Measurement of anthropometric parameters and biochemical analyses

The anthropometric measurements included FMP, weight and height. All instruments were validated according to the methods of the manufacturers. FMP was measured using a body composition analyser (InBody 720, Biospace Co, Ltd, Seoul, Korea). BMI was calculated as weight in kilograms divided by square of height in metres. Blood pressure was measured via auscultation using a standard clinical sphygmomanometer.

Levels of triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol and fasting plasma glucose were examined using an automatic biochemical analyser (Olympus chemistry analyser AU640, Olympus Optical Co, Ltd, Tokyo, Japan) using commercial kits (Olympus Optical Co, Ltd, Tokyo, Japan).

Measurement of sedentary behaviour and physical activity

A validated questionnaire was used to investigate sedentary behaviour and physical activity in children. The questionnaire referred to the questionnaire used in the study by Meng *et al.*¹¹ The questionnaires were completed by parents or guardians. The questions were retrospective and the questionnaire collected the data in the past 6 months. Sedentary behaviour was determined by the time spent either watching television or playing video/computer games per day in a week. Moderate physical activity (MPA) was determined by the time spent jogging or playing table tennis, or practising Tai Chi, etc, per week. High physical activity (HPA) was determined by the time spent playing football, basketball or badminton, etc, per week. HPA or MPA level was determined by the time spent on MPA or HPA per week. According to sedentary behaviour and physical activity, we stratified the participants into six groups, including sedentary behaviour <60 min/day, sedentary behaviour ≥ 60 min/day, HPA level <30 min/day, HPA

level ≥ 30 min/day, HPA or MPA level <150 min/week and HPA or MPA level ≥ 150 min/week.

DNA methylation analysis

Genomic DNA was extracted from peripheral white blood cells using a QIAamp DNA Blood Mini Kit (QIAGEN Inc, Valencia, California, USA).

The Sequenom MassARRAY platform (BioMiao Biological Technology, Beijing, China) was used to quantitatively examine the methylation of *FAIM2* promoter. The genomic DNA was bisulfite-treated using the EpiTect Bisulfite kit (Qiagen) according to the manufacturer's instructions. The target DNA region was amplified using the bisulfite-modified DNA. The specific primers are listed in online supplementary table S2. The primers were designed using epidesigner (<http://www.epidesigner.com>), and the methylation ratios were calculated using EpiTyper software V.1.0 (Sequenom, San Diego, California, USA).

Statistical analysis

The continuous variables are presented as the mean $\pm$ SD, and the categorical variables are presented as the percentages. Multiple linear regressions were used to investigate the difference in methylation levels between the different groups after adjusting for age and gender, or age, gender and BMI. A logistic regression model was used to investigate the interaction between the methylation levels of the *FAIM2* promoter and physical activity regarding the obesity status of the children (the model: obesity=CpG+PA+CpG*PA). The p value <0.05 was used to indicate statistically significant differences. We applied multiple testing to correct for multiple comparisons, the false discovery rate (FDR)¹² approach was used; FDR analysis (0.05 as criteria) was applied for six groups (sedentary behaviour <60 min/day, sedentary behaviour ≥ 60 min/day, HPA level <30 min/day, HPA level ≥ 30 min/day, HPA or MPA level <150 min/week and HPA or MPA level ≥ 150 min/week), and 36 CpG sites simultaneously (number of test: $36 \times 6 = 216$). In brief, if the original p value was less than p value for FDR, then it represented statistical significance; otherwise, it suggested non-statistical significance. Data were analysed using SPSS, V.13.0 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

The characteristics of the study participants are shown in online supplementary table S3. We analysed the associations between the methylation levels of the *FAIM2* promoter and the categories of sedentary behaviour or physical activity (see online supplementary tables S4–S6). There was no statistically significant difference after multiple testing. We analysed the associations between the methylation levels of the *FAIM2* promoter and sedentary behaviour in the cohort (table 1). The methylation levels at seven CpG sites showed significant differences between obese and lean participants, with

Table 1 Associations of the *FAIM2* promoter methylation with sedentary behaviour

CpG site	Sedentary behaviour (<60 min/day) (mean±SD)				Sedentary behaviour (≥60 min/day) (mean±SD)			
	Obese (n=8)	Lean (n=24)	p Value	β (95% CI)	Obese (n=38)	Lean (n=8)	p Value	β (95% CI)
-1319	87.50±3.25	86.92±2.57	0.59	0.012 (-0.032 to 0.055)	86.81±2.30	85.13±2.75	0.05	0.045 (-0.001 to 0.091)
-1172	96.75±4.37	89.38±11.22	0.30	0.006 (-0.006 to 0.018)	93.42±8.51	93.75±7.61	0.36	0.007 (-0.008 to 0.022)
-1157	87.86±4.70	89.83±2.71	0.004	-0.046 (-0.076 to -0.016)	88.72±7.89	85.63±7.63	0.64	0.003 (-0.011 to 0.018)
-1106	90.25±1.98	89.46±2.67	0.95	0.002 (-0.047 to 0.05)	88.72±4.10	86.00±5.48	0.36	0.012 (-0.015 to 0.039)
-1061, -1059	79.38±15.01	86.50±8.70	0.27	-0.006 (-0.017 to 0.005)	78.97±18.97	69.13±18.59	0.48	0.002 (-0.004 to 0.008)
-975	81.25±9.66	84.92±2.41	0.00004	-0.040 (-0.057 to -0.023))	84.83±2.25	83.50±2.56	0.41	0.023 (-0.033 to 0.078)
-931	95.13±1.89	96.88±2.17	0.14	-0.039 (-0.092 to 0.013)	96.20±3.74	94.38±6.12	0.69	0.006 (-0.023 to 0.034)
-878	67.63±7.46	67.33±4.38	0.15	0.017 (-0.006 to 0.039)	66.39±6.63	65.25±5.42	0.94	-0.001 (-0.019 to 0.018)
-859	75.13±6.36	78.29±3.65	0.12	-0.020 (-0.044 to 0.005)	74.75±9.33	72.50±5.81	0.93	-0.001 (-0.014 to 0.013)
-500	13.50±2.33	13.88±2.59	0.85	-0.005 (-0.05 to 0.043)	13.30±2.96	16.00±2.73	0.021	-0.041 (-0.075 to -0.006))
-413	5.38±5.68	3.00±0.88	0.00009	0.067 (0.037 to 0.097)	3.14±1.44	4.13±2.30	0.18	-0.046 (-0.113 to 0.021)
-399, -397, -390	6.63±4.27	6.46±3.65	0.56	-0.009 (-0.040 to 0.022)	7.68±3.54	9.50±3.85	0.57	-0.009 (-0.041 to 0.023)
-371	3.75±1.16	4.17±1.74	0.33	-0.035 (-0.107 to 0.038)	4.19±1.79	5.63±1.30	0.042	-0.062 (-0.121 to -0.002))
-362, -360	3.50±5.07	0.39±0.66	0.0006	0.065 (0.031 to 0.1)	1.40±2.01	1.29±1.60	0.72	-0.012 (-0.081 to 0.057)
-353, -349	6.25±6.45	1.96±0.86	0.00005	0.056 (0.032 to 0.08)	2.73±2.76	1.88±1.13	0.70	0.008 (-0.036 to 0.053)
-338, -336	12.25±3.81	10.08±2.02	0.028	0.046 (0.005 to 0.087)	11.43±3.27	14.50±4.24	0.16	-0.023 (-0.056 to 0.01)
-312, -307, -305	2.43±0.79	4.54±6.34	0.22	-0.010 (-0.025 to 0.006)	4.71±3.12	2.60±0.89	0.20	0.022 (-0.012 to 0.057)
-289	12.14±4.14	12.86±4.04	0.53	-0.007 (-0.029 to 0.015)	14.50±8.80	14.00±6.75	0.70	0.003 (-0.012 to 0.017)
-272, -269	11.43±2.51	18.25±13.03	0.17	-0.005 (-0.012 to 0.002)	21.29±14.82	16.17±16.85	0.28	0.004 (-0.003 to 0.011)
-259	11.43±3.36	16.08±5.69	0.044	-0.015 (-0.030 to 0)	16.75±7.52	11.00±3.74	0.08	0.012 (-0.002 to 0.026)
-225, -222, -219	1.57±1.62	1.75±5.14	0.56	0.006 (-0.014 to 0.026)	1.82±3.76	0.83±1.60	0.59	0.009 (-0.024 to 0.041)
-209	1.29±1.11	3.42±5.31	0.56	-0.005 (-0.024 to 0.013)	1.94±3.59	0.50±0.55	0.61	0.008 (-0.025 to 0.041)
-203, -201	7.57±4.96	10.25±13.26	0.10	-0.006 (-0.013 to 0.001)	13.42±11.42	10.83±8.82	0.76	0.002 (-0.009 to 0.012)
-195, -190	8.29±7.89	9.75±17.95	0.84	0.001 (-0.005 to 0.006)	20.31±24.14	9.17±12.78	0.26	0.003 (-0.002 to 0.007)
-183	5.14±1.21	5.42±4.25	0.86	0.002 (-0.021 to 0.025)	5.97±4.90	4.33±2.07	0.12	0.019 (-0.005 to 0.042)
-164	2.43±0.98	4.33±5.26	0.41	-0.007 (-0.026 to 0.011)	3.88±2.73	2.50±2.07	0.42	0.017 (-0.026 to 0.06)
-147, -145, -142	6.86±2.54	10.26±11.24	0.86	0.001 (-0.009 to 0.01)	14.94±13.90	6.20±5.07	0.25	0.004 (-0.003 to 0.012)
-134, -132, -129	2.57±1.40	5.21±9.36	0.60	0.003 (-0.008 to 0.014)	3.31±2.74	2.83±2.14	0.95	0.001 (-0.040 to 0.043)
-124	22.14±11.32	24.52±24.52	0.91	0.000 (-0.004 to 0.004)	30.56±22.11	12.17±16.41	0.24	0.003 (-0.002 to 0.008)
-115	5.14±1.21	5.42±4.25	0.86	0.002 (-0.021 to 0.025)	5.97±4.90	4.33±2.07	0.12	0.019 (-0.005 to 0.042)
-56	1.71±4.54	2.50±5.79	0.92	-0.001 (-0.018 to 0.016)	5.31±10.31	0.00±0.00	0.75	0.002 (-0.010 to 0.014)
-44, -36	8.86±1.77	9.08±8.16	0.93	0.001 (-0.012 to 0.013)	8.27±9.26	6.83±4.17	0.95	0.000 (-0.013 to 0.014)
-14, -7	5.86±3.63	4.92±5.96	0.12	-0.012 (-0.028 to 0.003)	4.00±3.29	6.00±6.96	0.60	-0.007 (-0.035 to 0.021)
19	3.86±1.35	4.83±4.22	0.55	-0.007 (-0.031 to 0.017)	4.36±2.04	4.33±2.07	0.63	-0.013 (-0.067 to 0.041)
54	32.29±9.39	27.83±15.59	0.41	0.003 (-0.004 to 0.009)	28.00±17.57	24.67±14.08	0.79	-0.001 (-0.008 to 0.006)
61	13.14±3.53	10.88±5.32	0.39	0.007 (-0.010 to 0.024)	13.56±5.03	15.17±5.27	0.16	-0.016 (-0.039 to 0.007)

p Values adjusted for age and gender. Those highlighted in bold indicate that the associations showed statistical significance.

Table 2 Associations between the methylation levels of the *FAIM2* promoter and high physical activity

CpG site	High physical activity level (<30 min/day) (mean±SD)				High physical activity level (≥30 min/day) (mean±SD)			
	Obese (n=32)	Lean (n=10)	p Value	β (95% CI)	Obese (n=12)	Lean (n=3)	p Value	β (95% CI)
-1319	86.90±2.57	87.00±1.76	0.69	0.011 (-0.043 to 0.064)	86.91±2.43	86.67±1.53	0.72	0.013 (-0.064 to 0.09)
-1172	94.03±8.80	96.60±5.64	0.62	0.004 (-0.013 to 0.021)	94.73±6.02	85.00±9.54	0.022	0.021 (0.004 to 0.038)
-1157	88.16±8.45	89.80±3.68	0.15	-0.012 (-0.029 to 0.005)	89.27±4.10	86.00±2.00	0.20	0.025 (-0.016 to 0.066)
-1106	88.94±3.71	88.30±5.40	0.90	-0.002 (-0.035 to 0.031)	89.09±4.68	88.33±5.03	0.34	0.016 (-0.020 to 0.052)
-1061, -1059	79.29±18.56	82.00±11.26	0.16	-0.005 (-0.013 to 0.002)	76.91±19.12	77.33±19.50	0.88	0.001 (-0.009 to 0.01)
-975	83.94±5.34	85.00±2.83	0.22	-0.017 (-0.045 to 0.011)	84.73±2.33	87.00±3.61	0.36	-0.027 (-0.090 to 0.036)
-931	95.80±4.00	97.00±2.62	0.025	-0.040 (-0.074 to -0.005)	96.36±1.96	95.33±4.51	0.72	0.011 (-0.056 to 0.079)
-878	66.58±7.00	67.10±4.15	0.56	-0.006 (-0.026 to 0.014)	66.36±5.32	72.00±5.00	0.12	-0.022 (-0.050 to 0.007)
-859	74.68±9.44	75.80±3.77	0.11	-0.013 (-0.028 to 0.003)	74.36±7.58	77.67±5.13	0.48	-0.008 (-0.032 to 0.016)
-500	13.10±3.06	12.80±3.12	0.77	0.006 (-0.035 to 0.047)	13.92±2.23	17.00±1.73	0.81	-0.009 (-0.088 to 0.07)
-413	3.52±3.16	3.50±1.43	0.42	0.018 (-0.027 to 0.064)	3.42±1.56	2.67±0.58	0.59	0.027 (-0.081 to 0.135)
-399, -397, -390	7.52±3.46	9.30±5.64	0.31	-0.016 (-0.048 to 0.016)	8.17±4.04	6.33±3.06	0.53	-0.013 (-0.056 to 0.03)
-371	4.03±1.56	4.80±1.99	0.16	-0.052 (-0.126 to 0.021)	4.08±2.02	4.67±2.08	0.55	-0.022 (-0.099 to 0.056)
-362, -360	1.46±2.85	0.44±1.01	0.26	0.031 (-0.024 to 0.086)	2.73±3.32	0.33±0.58	0.11	0.038 (-0.010 to 0.086)
-353, -349	2.87±2.95	1.80±1.14	0.11	0.037 (-0.009 to 0.083)	4.75±5.63	2.33±1.15	0.42	0.011 (-0.019 to 0.041)
-338, -336	11.35±3.17	12.30±3.89	0.76	0.006 (-0.036 to 0.049)	12.17±4.04	11.00±1.00	0.52	0.012 (-0.029 to 0.054)
-312, -307, -305	4.79±3.10	4.78±3.90	0.95	-0.001 (-0.041 to 0.039)	2.64±1.63	12.00±16.52	0.20	-0.013 (-0.036 to 0.009)
-289	14.90±9.24	11.75±4.80	0.39	0.006 (-0.008 to 0.021)	11.11±2.26	13.00±4.24	0.16	-0.050 (-0.126 to 0.026)
-272, -269	18.79±10.59	18.22±11.69	0.39	0.005 (-0.007 to 0.018)	22.00±20.94	30.67±17.56	0.38	-0.003 (-0.011 to 0.005)
-259	15.48±6.41	15.11±6.68	0.59	0.005 (-0.015 to 0.026)	14.50±6.56	21.33±6.66	0.37	-0.010 (-0.034 to 0.014)
-225, -222, -219	2.17±4.01	2.22±4.89	0.91	0.002 (-0.031 to 0.034)	0.73±1.27	0.00±0.00	0.19	0.087 (-0.051 to 0.225)
-209	1.97±3.18	3.44±6.19	0.10	-0.026 (-0.057 to 0.005)	1.83±3.95	0.00±0.00	0.80	0.005 (-0.039 to 0.049)
-203, -201	12.59±10.69	16.78±17.88	0.40	-0.004 (-0.015 to 0.006)	11.08±11.44	9.33±8.02	0.32	-0.007 (-0.022 to 0.008)
-195, -190	18.97±22.72	12.67±20.55	0.17	0.004 (-0.002 to 0.01)	16.33±24.26	4.67±2.52	0.48	-0.003 (-0.010 to 0.005)
-183	6.55±4.93	6.56±3.91	0.41	0.012 (-0.017 to 0.04)	4.75±3.14	4.67±2.52	0.59	0.013 (-0.039 to 0.065)
-164	4.07±2.70	3.89±3.79	0.70	-0.008 (-0.053 to 0.036)	2.64±2.01	8.67±13.28	0.14	-0.018 (-0.044 to 0.007)
-147, -145, -142	12.48±11.16	10.56±12.89	0.37	0.005 (-0.006 to 0.016)	15.08±17.58	3.50±0.71	0.70	-0.002 (-0.012 to 0.008)
-134, -132, -129	3.31±2.84	5.56±11.06	0.59	-0.006 (-0.030 to 0.017)	3.00±2.04	2.67±1.15	0.32	-0.047 (-0.144 to 0.051)
-124	30.07±23.72	20.13±12.47	0.52	0.002 (-0.004 to 0.008)	24.83±9.96	14.67±17.47	0.76	-0.002 (-0.017 to 0.013)
-115	6.55±4.93	6.56±3.91	0.41	0.012 (-0.017 to 0.04)	4.75±3.14	4.67±2.52	0.59	0.013 (-0.039 to 0.065)
-56	3.00±5.44	0.56±1.33	0.77	0.004 (-0.024 to 0.032)	4.67±7.82	0.67±1.15	0.94	0.001 (-0.022 to 0.024)
-44, -36	8.57±9.18	8.67±9.33	0.54	-0.004 (-0.019 to 0.01)	8.40±7.20	5.33±4.93	0.77	-0.004 (-0.032 to 0.025)
-14, -7	4.28±3.55	6.22±7.07	0.30	-0.014 (-0.042 to 0.013)	4.75±3.14	7.33±12.70	0.82	0.003 (-0.025 to 0.031)
19	4.34±2.04	3.33±1.87	0.80	-0.009 (-0.083 to 0.064)	4.33±1.83	9.00±7.94	0.25	-0.023 (-0.064 to 0.019)
54	26.93±13.52	32.11±11.62	0.14	-0.007 (-0.017 to 0.003)	33.00±21.88	21.00±27.71	0.88	-0.001 (-0.008 to 0.007)
61	12.93±5.04	12.22±7.77	0.71	-0.004 (-0.028 to 0.019)	14.82±4.12	8.67±4.93	0.037	0.031 (0.002 to 0.06)

p Values adjusted for age and gender. Those highlighted in bold indicate that the associations showed statistical significance.

Table 3 Associations between the methylation levels of the *FAIM2* promoter and high or moderate physical activity

CpG site	High or moderate physical activity level (<150 min/week) (mean $\pm$ SD)				High or moderate physical activity level ($\geq$ 150 min/week) (mean $\pm$ SD)			
	Obese (n=28)	Lean (n=15)	p Value	β (95% CI)	Obese (n=15)	Lean (n=13)	p Value	β (95% CI)
-1319	86.93 $\pm$ 2.53	85.93 $\pm$ 2.46	0.09	0.046 (-0.007 to 0.099)	86.79 $\pm$ 2.67	85.62 $\pm$ 1.66	0.15	0.044 (-0.017 to 0.104)
-1172	94.63 $\pm$ 9.22	90.40 $\pm$ 11.85	0.09	0.011 (-0.002 to 0.024)	94.00 $\pm$ 5.57	89.69 $\pm$ 10.56	0.10	0.013 (-0.003 to 0.029)
-1157	87.33 $\pm$ 8.80	87.20 $\pm$ 5.89	0.59	-0.005 (-0.022 to 0.013)	90.00 $\pm$ 3.72	89.69 $\pm$ 3.04	0.71	0.008 (-0.035 to 0.05)
-1106	88.74 $\pm$ 3.81	87.53 $\pm$ 4.41	0.92	0.002 (-0.034 to 0.038)	89.21 $\pm$ 4.32	88.77 $\pm$ 3.27	0.51	0.012 (-0.025 to 0.05)
-1061, -1059	78.89 $\pm$ 19.44	78.27 $\pm$ 16.77	0.70	-0.001 (-0.009 to 0.006)	78.29 $\pm$ 18.10	82.00 $\pm$ 13.31	0.53	-0.003 (-0.012 to 0.006)
-975	83.48 $\pm$ 5.69	84.00 $\pm$ 2.62	0.16	-0.021 (-0.051 to 0.008)	85.07 $\pm$ 1.54	84.92 $\pm$ 2.93	0.91	0.004 (-0.065 to 0.073)
-931	95.69 $\pm$ 4.29	95.00 $\pm$ 4.78	0.72	-0.006 (-0.038 to 0.027)	96.36 $\pm$ 1.74	97.23 $\pm$ 2.62	0.55	-0.019 (-0.085 to 0.047)
-878	66.30 $\pm$ 7.29	65.40 $\pm$ 4.39	0.64	0.005 (-0.017 to 0.026)	67.50 $\pm$ 5.98	67.85 $\pm$ 4.69	0.23	-0.016 (-0.043 to 0.011)
-859	74.48 $\pm$ 9.98	73.87 $\pm$ 5.59	0.34	-0.008 (-0.025 to 0.009)	74.71 $\pm$ 7.17	78.38 $\pm$ 3.57	0.36	-0.011 (-0.036 to 0.013)
-500	13.19 $\pm$ 2.91	14.33 $\pm$ 2.99	0.23	-0.027 (-0.073 to 0.018)	13.47 $\pm$ 2.85	14.08 $\pm$ 2.78	0.50	-0.016 (-0.065 to 0.032)
-413	3.59 $\pm$ 3.32	3.07 $\pm$ 1.03	0.11	0.041 (-0.009 to 0.091)	3.20 $\pm$ 1.66	3.23 $\pm$ 1.01	0.61	-0.025 (-0.126 to 0.075)
-399, -397, -390	7.44 $\pm$ 3.49	7.00 $\pm$ 3.98	0.47	0.014 (-0.025 to 0.052)	7.80 $\pm$ 4.18	7.69 $\pm$ 4.17	0.66	-0.007 (-0.041 to 0.026)
-371	4.19 $\pm$ 1.36	5.07 $\pm$ 2.09	0.12	-0.063 (-0.143 to 0.017)	3.87 $\pm$ 2.13	4.31 $\pm$ 0.95	0.16	-0.055 (-0.134 to 0.024)
-362, -360	1.71 $\pm$ 2.90	0.62 $\pm$ 0.96	0.23	0.036 (-0.024 to 0.095)	2.25 $\pm$ 3.39	0.50 $\pm$ 0.67	0.19	0.039 (-0.020 to 0.098)
-353, -349	2.96 $\pm$ 3.11	1.93 $\pm$ 1.03	0.06	0.048 (-0.002 to 0.099)	4.07 $\pm$ 5.23	1.85 $\pm$ 0.99	0.40	0.015 (-0.020 to 0.049)
-338, -336	11.37 $\pm$ 3.47	12.07 $\pm$ 3.69	0.44	0.017 (-0.027 to 0.06)	12.20 $\pm$ 3.45	10.31 $\pm$ 2.50	0.67	0.010 (-0.036 to 0.055)
-312, -307, -305	4.36 $\pm$ 2.83	3.77 $\pm$ 3.35	0.61	0.012 (-0.034 to 0.058)	3.00 $\pm$ 1.66	5.42 $\pm$ 8.35	0.53	-0.008 (-0.034 to 0.018)
-289	14.44 $\pm$ 9.73	13.08 $\pm$ 3.86	0.55	0.005 (-0.012 to 0.022)	12.83 $\pm$ 4.45	13.70 $\pm$ 5.74	0.81	0.004 (-0.030 to 0.038)
-272, -269	18.48 $\pm$ 10.42	14.46 $\pm$ 6.44	0.023	0.016 (0.002 to 0.03)	21.00 $\pm$ 19.57	24.00 $\pm$ 17.99	0.83	-0.001 (-0.008 to 0.007)
-259	15.12 $\pm$ 6.78	16.15 $\pm$ 5.65	0.87	0.002 (-0.020 to 0.024)	15.87 $\pm$ 6.09	15.77 $\pm$ 5.88	0.63	0.006 (-0.019 to 0.03)
-225, -222, -219	1.84 $\pm$ 3.22	1.38 $\pm$ 4.11	0.48	0.014 (-0.026 to 0.055)	0.57 $\pm$ 0.85	2.38 $\pm$ 5.75	0.62	-0.009 (-0.046 to 0.028)
-209	2.60 $\pm$ 4.05	1.23 $\pm$ 1.64	0.92	-0.002 (-0.046 to 0.042)	0.60 $\pm$ 0.99	3.08 $\pm$ 5.24	0.46	-0.014 (-0.051 to 0.024)
-203, -201	11.72 $\pm$ 10.16	10.38 $\pm$ 13.59	0.78	0.002 (-0.011 to 0.015)	10.53 $\pm$ 10.51	11.85 $\pm$ 12.54	0.35	-0.006 (-0.018 to 0.007)
-195, -190	18.16 $\pm$ 22.58	9.46 $\pm$ 17.36	0.036	0.007 (0 to 0.013)	13.73 $\pm$ 22.16	11.69 $\pm$ 19.14	0.58	-0.002 (-0.009 to 0.005)
-183	6.56 $\pm$ 4.98	5.15 $\pm$ 2.85	0.011	0.040 (0.01 to 0.07)	4.40 $\pm$ 2.85	5.85 $\pm$ 5.11	0.32	-0.017 (-0.050 to 0.017)
-164	3.88 $\pm$ 2.47	2.69 $\pm$ 1.70	0.29	0.032 (-0.028 to 0.092)	2.71 $\pm$ 1.94	4.85 $\pm$ 6.78	0.26	-0.016 (-0.045 to 0.013)
-147, -145, -142	12.16 $\pm$ 11.49	9.54 $\pm$ 10.49	0.18	0.008 (-0.004 to 0.021)	14.00 $\pm$ 15.78	11.91 $\pm$ 11.84	0.69	-0.002 (-0.013 to 0.009)
-134, -132, -129	2.76 $\pm$ 1.69	5.54 $\pm$ 9.16	0.40	-0.011 (-0.037 to 0.015)	3.00 $\pm$ 1.81	5.15 $\pm$ 9.12	0.77	-0.003 (-0.026 to 0.019)
-124	26.84 $\pm$ 20.58	28.00 $\pm$ 26.02	0.66	-0.001 (-0.008 to 0.005)	25.07 $\pm$ 9.72	17.23 $\pm$ 20.56	0.72	0.002 (-0.008 to 0.012)
-115	6.56 $\pm$ 4.98	5.15 $\pm$ 2.85	0.011	0.040 (0.01 to 0.07)	4.40 $\pm$ 2.85	5.85 $\pm$ 5.11	0.32	-0.017 (-0.050 to 0.017)
-56	3.60 $\pm$ 5.68	0.85 $\pm$ 1.41	0.86	0.003 (-0.029 to 0.035)	4.00 $\pm$ 7.24	1.15 $\pm$ 3.60	0.72	0.004 (-0.021 to 0.03)
-44, -36	8.92 $\pm$ 9.77	5.92 $\pm$ 4.31	0.65	0.004 (-0.013 to 0.021)	7.62 $\pm$ 6.75	13.23 $\pm$ 9.05	0.14	-0.013 (-0.030 to 0.004)
-14, -7	3.64 $\pm$ 2.25	4.85 $\pm$ 5.61	0.29	-0.020 (-0.057 to 0.017)	5.53 $\pm$ 4.56	5.85 $\pm$ 7.50	0.42	0.009 (-0.014 to 0.033)
19	4.52 $\pm$ 2.06	4.54 $\pm$ 2.76	0.13	-0.049 (-0.114 to 0.016)	4.07 $\pm$ 1.87	4.92 $\pm$ 4.82	0.50	-0.014 (-0.055 to 0.028)
54	28.56 $\pm$ 17.76	25.23 $\pm$ 14.50	0.52	-0.003 (-0.012 to 0.006)	29.43 $\pm$ 14.96	25.75 $\pm$ 16.68	0.70	0.002 (-0.008 to 0.012)
61	12.92 $\pm$ 4.37	11.31 $\pm$ 6.99	0.47	0.009 (-0.017 to 0.036)	14.43 $\pm$ 5.65	11.92 $\pm$ 4.77	0.59	-0.008 (-0.037 to 0.022)

p Values adjusted for age and gender. Those highlighted in bold indicate that the associations showed statistical significance.

sedentary behaviour <60 min/day, especially the methylation levels at site -975, site -413, sites -362 and -360, and sites -353 and -349 ($p=0.00004$, 0.00009 , 0.0006 and 0.00005 , respectively), after adjusting for age and gender. We conducted multiple testing and these four CpG sites reached significance. The methylation levels at two CpG sites (site -500 and site -371) showed significant differences between obese and lean participants with sedentary behaviour ≥ 60 min/day, but these two CpG sites did not reach significance after multiple testing.

Tables 2 and 3 show the associations between the methylation levels of the *FAIM2* promoter and physical activity. There were significant differences between the methylation levels at four CpG sites in obese and lean participants with HPA or MPA level <150 min/week, but these four CpG sites did not reach significance after multiple testing. There was no statistically significant difference between obese and lean participants with HPA or MPA level ≥ 150 min/week.

There was no statistically significant interaction between the methylation levels of the *FAIM2* promoter and physical activity regarding obesity status after multiple testing (see online supplementary table S7).

DISCUSSION

Previous studies have shown that lifestyle factors might modify epigenetic patterns³ and the methylation levels of the *FAIM2* promoter are significantly associated with obesity,¹⁰ but whether the molecular mechanisms by which *FAIM2* affects obesity are involved in lifestyle is unclear. In this study, we investigated the potential associations of the *FAIM2* promoter methylation with sedentary behaviour and physical activity in obese and lean children. The methylation levels of the *FAIM2* promoter were significantly different in obese and lean children when the groups were stratified by sedentary behaviour and physical activity. There were significant differences in the associations between the *FAIM2* promoter methylation with sedentary behaviour and physical activity between obese and lean participants. Our results suggest that lifestyle may possibly be mediating the process of the *FAIM2* involved in obesity.

FAIM2 can bind directly to the Fas receptor to protect cells against apoptosis,^{5 13} which is a nervous system-specific inhibitor of Fas/CD95-mediated apoptosis. GWA studies in Caucasians and subsequently replicated studies in Asian populations showed that the SNP rs7138803 near *FAIM2* was significantly associated with obesity.⁶⁻⁸ Recently, it was reported that the expression of *FAIM2* was regulated by high fat feeding in the brain,¹⁴ and that the methylation levels of the *FAIM2* promoter were significantly associated with obesity.¹⁰

Increasing evidence shows that lifestyle factors may affect epigenetic alterations, especially DNA methylation, which has been identified to be associated with a variety of human diseases including obesity.^{3 4 15}

For example, physical activity is associated with changes in DNA methylation at many genes related to adipocyte metabolism and type 2 diabetes.^{16 17}

This study may not provide direct evidence that the expression of *FAIM2* influences obesity, because of the lack of gene expression data from leucocytes and tissues, but our study demonstrated the differences of the associations of the *FAIM2* promoter methylation with sedentary behaviour and physical activity between obese and lean children.

There are a few limitations to this study. First, the age range of the obese and lean participants in this study is wide and the sample size is small. Studies with greater sample sizes are needed to examine the associations. Second, obese participants were on average 3.5 years older than lean participants. We want to eliminate the effect by adjusting for age. It is important to adjust the stage of puberty in the analyses, so we will collect data on the stage of puberty in future studies. Third, the data of sedentary behaviour and physical activity were collected by questionnaires. The reliability of questionnaires depends on good communication between the parents and children. Fourth, there were no gene expression data from leucocytes or tissues in the study. Previous studies showed the expression of *FAIM2* in the hypothalamus or some other tissues, but there were no expression data of this gene in peripheral blood leucocytes. Fifth, our study investigated the methylation levels in peripheral blood leucocytes, but not in the hypothalamus or adipocytes.

CONCLUSIONS

In this study we explored, for the first time, the significant differences in the associations between the *FAIM2* promoter methylation with sedentary behaviour and physical activity between obese and lean children. Although the function of *FAIM2* in the brain remains to be fully researched, identification of methylation changes affected by lifestyle factors will provide important potential to develop new therapies to prevent obesity.

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Contributors LW designed the study and wrote the manuscript. LW and GH collected the data. YS performed the statistical analysis. XZ, MZ, YY, DH, LM, JL and HC collected the DNA samples. All the authors reviewed the manuscript. LW and XZ contributed equally to this work. JM directed the project.

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Competing interests None declared.

Patient consent Obtained.

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