

# 385 C/A polymorphism of the fatty acid amide hydrolase gene is associated with metabolic syndrome in the Chinese Han population

JinYang Zeng, JianRong Li, GuoLiang Huang

Department of Endocrinology, Union Hospital, Fujian Medical University, and Fujian Institute of Endocrinology, Fuzhou, P. R. China

**Submitted:** 13 October 2010

**Accepted:** 26 December 2010

Arch Med Sci 2011; 7, 3: 423-427

DOI: 10.5114/aoms.2011.23406

Copyright © 2011 Termedia & Banach

## Corresponding author:

Guoliang Huang MD, PhD

Department of Endocrinology

Union Hospital, Fujian

Medical University

and Fujian Institute

of Endocrinology

29 Xin-Quan Road

Fuzhou, 350001, P. R. China

Phone/fax:

+86 0591 87966136

E-mail:

guolianghuang@126.com

## Abstract

**Introduction:** The endocannabinoid system participates in food intake, energy balance and lipid and glucose metabolism. The biological effects of cannabinoids are limited by the activation of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH). This study aims to analyse whether 385 C/A polymorphism of FAAH is associated with metabolic syndrome (MetS) in the Chinese Han population.

**Material and methods:** A total of 112 subjects at risk for MetS and 80 healthy controls from Fuzhou, China were genotyped for 385 C/A polymorphism of FAAH using TaqMan assay. Anthropometric measurements and biochemical assessments such as BMI, waist circumference, blood pressure, serum triglycerides (TG), serum total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, fasting plasma glucose, and plasma insulin levels were performed.

**Results:** CA and AA genotypes of FAAH had higher incidence in MetS subjects than in control subjects. CA and AA genotypes of FAAH in subjects with MetS had relatively elevated levels of waist circumference, body mass index, homeostasis model assessment of insulin resistance (HOMA-IR) and serum triglycerides, and lowered level of high-density lipoprotein cholesterol (HDL-c) compared with CC genotype in MetS subjects.

**Conclusions:** Results suggest that 385 C/A polymorphism of the FAAH gene may confer an increased risk of MetS in the Chinese Han population.

**Key words:** polymorphism, fatty acid amide hydrolase, metabolic syndrome.

## Introduction

Metabolic syndrome (MetS) is characterized by multiple compounding factors such as abdominal obesity, insulin resistance, elevated serum triglyceride (TG) levels, low high-density lipoprotein cholesterol (HDL-c) levels and high blood pressure [1]. Many susceptibility genes have been shown to contribute to the susceptibility of developing MetS [2].

The endocannabinoid system plays an important role in the regulation of food consumption and energy balance; it also influences the regulation of lipid and glucose metabolism [3]. The main enzyme that inactivates the endocannabinoid is fatty acid amide hydrolase (FAAH), which has been identified as a catabolic enzyme capable of degrading most of the endocannabinoids [4]. The endocannabinoid level was significantly elevated

in mice lacking FAAH, which indicates that FAAH is a key regulator of endocannabinoid signalling [5].

A common C > A single nucleotide polymorphism (SNP) resulting in a missense mutation (385C > A) has been described previously. An association of the mutant genotype of FAAH with a worse cardiovascular profile, such as increased weight, body mass index (BMI), waist circumference (WC), insulin and TNF- $\alpha$  levels, and decreased adiponectin levels was also observed [6]. This study suggests that FAAH plays a great role in the metabolic aspects of feeding behaviour and body weight.

Although studies have focused on the association of the C385A polymorphism of FAAH with obesity and diabetes, an investigation on the association of this polymorphism with MetS has not been performed yet. Therefore, this study aims to analyse whether 385 C/A polymorphism of FAAH is associated with MetS in the Chinese Han population.

## Material and methods

### Subjects

A total of 112 MetS subjects undergoing an annual physical examination in the outpatient department of Union Hospital, Fujian Medical University were enrolled in this study. Metabolic syndrome was defined using the criteria established in the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment in the Asian population (Adult Treatment Panel III) as the presence of 3 or

more of the following risk factors [7]: WC  $\geq$  80 cm in women or  $\geq$  90 cm in men, TG  $\geq$  1.69 mmol/l, HDL-c  $\leq$  1.29 mmol/l in women or  $\leq$  1.03 mmol/l in men, systolic blood pressure (SBP)  $\geq$  130 mmHg and/or diastolic blood pressure (DBP)  $\geq$  85 mmHg, fasting blood glucose (FBG)  $\geq$  5.6 mmol/l.

Normal subjects ( $n = 80$ ) who entered the healthy evaluation programme were assigned as the control group for this study. These subjects had none of the five criteria of MetS described above and no history of obesity, dyslipidaemia, hypertension, or diabetes mellitus. This study was approved by the ethics committee of our hospital, and informed consent was obtained from all participants.

### Measurements

Anthropometric (height, weight, WC and blood pressures) and laboratory analyses were performed. Fasting plasma glucose, serum TG, total cholesterol (TC), HDL-c and low-density lipoprotein cholesterol (LDL-c) were detected using an auto biochemistry instrument (LX20, BECMAN, CA, USA). Plasma fasting insulin level was measured by radioimmunoassay (RIA Diagnostic Corporation, Los Angeles, CA). Body mass index was calculated as weight in kilograms divided by the square of height in metres ( $\text{kg}/\text{m}^2$ ). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the insulin reading ( $\mu\text{IU}/\text{ml}$ ) multiplied by plasma glucose level ( $\text{mmol}/\text{l}$ ) and divided by 22.5.

### DNA genotyping

Venous blood of all subjects was collected and frozen immediately at  $-20^\circ\text{C}$  until the extraction of genomic DNA from nucleated white blood cells. DNA was isolated from 200 ml of anticoagulated peripheral blood using a commercially available kit according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit, QIAGEN Inc, CA, USA). The 385 C/A polymorphism of the FAAH gene was determined using the TaqMan SNP Genotyping Assays on ABI 7900 (Applied Biosystems, Foster City, CA, USA). As a standard laboratory quality control measure, 10% of DNA samples were randomly selected and systematically re-genotyped. A concordance rate of 100% was recorded for this SNP.

### Statistical analysis

Data are displayed as means  $\pm$  standard deviation (SD). Student's *t*-test was performed to compare biochemical variables between the MetS and control group.  $\chi^2$  and *p* values for FAAH 385 C/A genotypes among MetS patients and controls were calculated using an online  $\chi^2$  test. The different variables of FAAH genotypes among MetS patients

**Table I.** Comparison of anthropometric and biological parameters between MetS patients and control subjects

Characteristics	MetS group	Control group	Value of <i>p</i>
Age [years]	60.04 $\pm$ 9.46	57.98 $\pm$ 10.19	0.150
Gender (M/F)	65/47	45/35	0.805
WC [cm]	84.63 $\pm$ 10.05	78.36 $\pm$ 7.82	< 0.001
BMI [ $\text{kg}/\text{m}^2$ ]	27.06 $\pm$ 3.86	24.19 $\pm$ 2.45	< 0.001
SBP [mmHg]	142.13 $\pm$ 12.74	123.81 $\pm$ 14.34	< 0.001
DBP [mmHg]	84.42 $\pm$ 9.58	82.00 $\pm$ 8.97	0.078
HOMA-IR	2.48 $\pm$ 0.77	1.56 $\pm$ 0.48	< 0.001
TG [ $\text{mmol}/\text{l}$ ]	1.80 $\pm$ 0.85	1.07 $\pm$ 0.51	< 0.001
TC [ $\text{mmol}/\text{l}$ ]	5.13 $\pm$ 1.12	5.15 $\pm$ 0.89	0.910
HDL-c [ $\text{mmol}/\text{l}$ ]	1.13 $\pm$ 0.24	1.41 $\pm$ 0.22	< 0.001
LDL-c [ $\text{mmol}/\text{l}$ ]	3.35 $\pm$ 0.83	3.24 $\pm$ 0.58	0.468

Values are given as mean  $\pm$  SD.

MetS – metabolic syndrome, WC – waist circumference, BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, HOMA-IR – homeostasis model assessment of insulin resistance, TG – triglycerides, TC – total cholesterol, HDL-c – high-density lipoprotein cholesterol, LDL-c – low-density lipoprotein cholesterol

**Table II.** Genotype and allele frequencies of the 385 C/A polymorphism of FAAH between MetS subjects and control subjects

Group	n	Genotype			Allele	
		CC	CA	AA	C	A
MetS	112	68 (60.7%)	40 (35.7%)	4 (3.6%)	78.6%	21.4%
Control	80	63 (78.8%)	16 (20.0%)	1 (1.2%)	88.8%	11.2%

Pearson  $\chi^2$  value = 8.567,  $p$  = 0.014, n – sample number, other abbreviations – see table I

were analysed using Student's *t*-test. The normality of distributions of the variables was tested by the Kolmogorov-Smirnov (KS) test. A *p* value under 0.05 was considered statistically significant.

## Results

The anthropometric and biological parameters of the MetS patients and the control subjects are presented in Table I. The MetS group showed higher levels of WC, BMI, SBP, HOMA-IR and serum TG and a lowered HDL-c level compared with the control group. No significant differences were observed in age, gender, DBP, serum TC and LDL-c levels between the two groups.

Genotype and allele frequencies of the FAAH gene in MetS subjects and healthy controls are listed in Table II. A significantly different allelic distribution of FAAH gene polymorphism was found between the two groups. A greater proportion of the CA and AA genotypes and a lower proportion of the CC genotype were observed in the MetS group compared with the healthy controls.

Patients with the CC genotype had significantly lower levels of WC, BMI, HOMA-IR and serum TG, and increased HDL-c levels than those with the CA and AA genotypes in the MetS group. No significant differences were found in SBP, DBP, serum TC and LDL-c levels between the mutant-type group and wild-type group. The characteristics of the different genotypes of the FAAH 385 C/A polymorphism in MetS subjects are presented in Table III.

## Discussion

The present study suggests that the FAAH gene may constitute a predisposing factor to MetS. Previous studies have described the association of this polymorphism with obesity and lipid metabolism. However, there is no comprehensive investigation about the possible role of this polymorphism in the development of MetS. In our study, a greater proportion of the CA and AA genotypes, and a smaller proportion of the CC genotype, were found in the MetS patients compared with the healthy group. Our results reveal that the FAAH gene may be an important candidate gene of MetS.

The possible association between the 385 C/A polymorphism of the FAAH and MetS can be

explained by the biological role of FAAH. The endocannabinoid system plays an important role in the hypothalamic and peripheral regulation of food intake, obesity and metabolism. Fatty acid amide hydrolase serves as a key enzyme in the endocannabinoid degradation pathway by degrading most of the endocannabinoids [8]. Therefore, it is plausible to hypothesize that mutation in the FAAH gene may cause decreased expression or activity of FAAH. This leads to increased stimulation of the endocannabinoid receptor and thereby results in increased food intake and body fat accumulation. Significantly elevated levels of the endocannabinoid were found in subjects with the mutant-type of the FAAH gene than in those with wild-type controls, which indicated that the endocannabinoid system activation may be due to the effect of the FAAH mutant genotype on the plasma endocannabinoid levels [9]. FAAH<sup>-/-</sup> mice showed boosted energy storage compared with their wild-type littermates [10]. These results suggest the possible role of FAAH in the regulation of food intake and energy balance.

Previous studies have shown the association between the 385 C/A polymorphism of FAAH and obesity. The homozygous FAAH 385 A/A genotype was significantly associated with overweight and obesity in white subjects and in black subjects but not in a small group of Asians [11]. An association of the FAAH variants at the early onset of obesity was also observed [12]. An elevated level of adipose

**Table III.** Comparison of characteristics of different genotypes of FAAH gene in subjects with MetS

Characteristics	CC	CA + AA	Value of <i>p</i>
WC	82.94 ±10.68	87.23 ±8.44	0.020
BMI	26.34 ±3.82	28.17 ±3.69	0.014
SBP	141.69 ±11.45	142.80 ±14.64	0.673
DBP	84.63 ±9.28	84.09 ±10.13	0.772
HOMA-IR	2.34 ±0.74	2.68 ±0.79	0.023
TG	1.67 ±0.84	2.02 ±0.83	0.033
TC	5.06 ±1.21	5.24 ±0.97	0.409
HDL-c	1.16 ±0.26	1.07 ±0.18	0.043
LDL-c	3.29 ±0.89	3.34 ±0.72	0.721

Values are given as mean ± SD, abbreviations – see table I

FAAH was shown to be significantly associated with a decrease in food intake in C3H mice [13]. These findings indicate that FAAH variants may be involved in the aetiology of obesity. Similarly, the results of this study also demonstrate that WC and BMI were increased more in the mutant-type group than in the wild-type group.

The present study demonstrates that the level of HOMA-IR was significantly elevated in the subjects carrying A allele in comparison with the wild control subjects, indicating the possible involvement of FAAH in the development of insulin resistance. Similar results were also reported by other studies. Compared with the controls,  $\text{FAAH}^{-/-}$  mice showed increased plasma insulin and blood glucose levels, leading to enhanced insulin resistance [10]. A higher plasma insulin level was found in the mutant-type group than in the wild-type group [6]. However, another study reported that insulin, glucose and HOMA levels are elevated more in the wild-type group than in the mutant-type group [14]. This discrepancy may have resulted from the environmental and ethnic differences between the populations or the differences in sample collection and storage.

This study suggests that the mutant genotype of the FAAH gene in subjects with MetS had more elevated TG levels and lower HDL-c concentrations than the wild genotype. Previous evidence suggests that FAAH may be involved in the pathogenesis of dyslipidaemia. The 385 C/A polymorphism of the FAAH gene was found to be associated with increased serum TG and reduced levels of HDL-c [15]. Plasma free fatty acids and TG concentrations were significantly increased in  $\text{FAAH}^{-/-}$  mice compared to the normal littermates [10]. These results suggest a possible involvement of FAAH activation in the metabolism of obesity-related hypertriglyceridaemia and predict the potential efficacy of cannabinoid antagonists in treating metabolic diseases.

A previous study suggests that young male subjects carrying the FAAH variant have relatively lower blood pressure [16]. We compared the SBP and the DBP between the MetS subjects from the wild-type and those from the mutant-type group. Blood pressure was not correlated with this polymorphism, a finding that is distinct from the study of Sarzani [16]. No significant relationship of blood pressure with the 385 C/A polymorphism of FAAH was also reported by several other investigators [6, 17]. This inconsistency may be attributed to the complex interactions between multiple population-specific genetic factors as well as environmental factors.

The potential limitations of these data merit consideration. First, this cross-sectional study has a relatively small population size. Therefore, it is necessary to validate our data in prospective

studies with a larger population size. Second, as our study was conducted in a sample of Chinese patients, extrapolation of the data to other ethnic groups should be done with great caution.

In conclusion, this study indicates that the 385 C/A polymorphism of the FAAH gene may be a risk factor for susceptibility to MetS. Thus, people with this polymorphism who are at a high risk for MetS should take necessary precautions such as doing more exercises and eating healthy food to prevent or delay the development of MetS. This finding may support the therapeutic use of cannabinoid antagonist strategies.

## References

- Kostapanos MS, Lianis GL, Elisaf M. Features of the metabolic syndrome relating to cardiorenal outcomes. *Arch Med Sci* 2008; 4: 424-6.
- Bruce KD, Hanson MA. The developmental origins, mechanisms, and implications of metabolic syndrome. *J Nutr* 2010; 140: 648-52.
- Stefano GB, Kream RM. Mini-symposium on food vulnerability in Society: Sentinel Chemical Messengers: endocannabinoid signaling transcends pain. *Arch Med Sci* 2009; 5: 602-12.
- Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). *Prostaglandins Leukot Essent Fatty Acids* 2002; 66: 201-10.
- Cravatt BF, Demarest K, Patricelli MP, et al. Super-sensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A* 2001; 98: 9371-6.
- de Luis DA, Sagrado MG, Aller R, Izaola O, Conde R, Romero E. C358A missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) and insulin resistance in patients with diabetes mellitus type 2. *Diabetes Res Clin Pract* 2010; 88: 76-80.
- Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA* 2001; 285: 2486-97.
- Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 2003; 4: 873-84.
- Sipe JC, Scott TM, Murray S, et al. Biomarkers of endocannabinoid system activation in severe obesity. *PLoS One* 2010; 5: e8792.
- Touriño C, Oveisi F, Lockney J, Piomelli D, Maldonado R. FAAH deficiency promotes energy storage and enhances the motivation for food. *Int J Obes* 2010; 34: 557-68.
- Sipe JC, Waalen J, Gerber A, Beutler E. Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). *Int J Obes* 2005; 29: 755-9.
- Müller TD, Brönnér G, Wandolski M, et al. Mutation screen and association studies for the fatty acid amide hydrolase (FAAH) gene and early onset and adult obesity. *BMC Med Genet* 2010; 11: 2.
- Thabuis C, Destaillats F, Landrier JF, Tissot-Favre D, Martin JC. Analysis of gene expression pattern reveals potential targets of dietary oleylethanolamide in reducing body fat gain in C3H mice. *J Nutr Biochem* 2010; 21: 922-8.

14. de Luis DA, Gonzalez Sagrado M, Aller R, Izaola O, Conde R, Romero E. C358A missense polymorphism of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) and visfatin levels in obese females. *Int J Obes* 2010; 34: 511-5.
15. Zhang Y, Sonnenberg GE, Baye TM, et al. Obesity-related dyslipidemia associated with FAAH, independent of insulin response, in multigenerational families of Northern European descent. *Pharmacogenomics* 2009; 10: 1929-39.
16. Sarzani R, Bordicchia M, Salvi F, et al. A human fatty acid amide hydrolase (FAAH) functional gene variant is associated with lower blood pressure in young males. *Am J Hypertens* 2008; 21: 960-3.
17. Papazoglou D, Panagopoulos I, Papanas N, et al. The fatty acid amide hydrolase (FAAH) Pro129Thr polymorphism is not associated with severe obesity in Greek subjects. *Horm Metab Res* 2008; 40: 907-10.