



## Caveolin-1 genetic polymorphism interacts with PUFA to modulate metabolic syndrome risk

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### Abstract

Several studies have reported a significant association between the metabolic syndrome (MetS) and mortality around the world. Caveolin-1 (CAV-1) has been widely studied in dyslipidaemia, and several studies have indicated that CAV-1 genetic variations may correlate with dietary intake of fatty acids. This study aimed to investigate the interaction of CAV-1 rs3807992 with types of dietary fatty acid in the MetS risk. This cross-sectional study was carried out on 404 overweight and obese females. Dietary intake was obtained from a 147-item FFQ. The CAV-1 genotype was measured using the PCR-restriction fragment length polymorphism method. Anthropometric values and serum levels (TC, LDL, HDL, TAG and FBS) were measured by standard methods. It was observed that the (AA + AG) group had significantly higher BMI, waist circumference and DBP ( $P = 0.02$ ,  $P = 0.02$ , and  $P = 0.01$ , respectively) and lower serum LDL, HDL and TC ( $P < 0.05$ ) than the GG group. It was found that A allele carriers were at higher odds of the MetS ( $P = 0.01$ ), abdominal obesity ( $P = 0.06$ ), increased TAG concentration ( $P = 0.01$ ), elevated blood pressure (BP) ( $P = 0.01$ ), increased glucose concentration ( $P = 0.45$ ) and decreased HDL-cholesterol concentration ( $P = 0.03$ ). Moreover, the interaction of CAV-1 and SFA intake was significant in terms of the MetS ( $P = 0.03$ ), LDL ( $P = 0.03$ ) and BP ( $P = 0.01$ ). Additionally, the (AA + AG) group was significantly related to PUFA intake in terms of the MetS ( $P = 0.04$ ), TAG ( $P = 0.02$ ), glucose ( $P = 0.02$ ) and homoeostasis model assessment insulin resistance ( $P = 0.01$ ). Higher PUFA consumption might attenuate the CAV-1 rs3807992 associations with the MetS, and individuals with greater genetic predisposition appeared to have a higher risk of the MetS, associated with higher SFA consumption.

**Key words:** Caveolin-1: Dietary fat intakes: Gene–diet interaction: Metabolic syndrome: Obesity

The metabolic syndrome (MetS) is related to metabolic abnormalities including obesity, hyperglycaemia, dyslipidaemia and hypertension and is prevalent worldwide<sup>(1)</sup>. Compared with people without the MetS, people with MetS had a higher overall number of deaths for all causes<sup>(2)</sup>. Particularly, the evidence demonstrates that genetic variations play a main part in the prevention and treatment of various chronic diseases, particularly in the MetS<sup>(3–5)</sup>. The MetS and CVD also demonstrate the role of genetic and environmental factors in diet-related disorders. The genetic background of metabolic disorders has been shown to relate to death rate over the last few decades<sup>(6)</sup>. There is therefore a need to identify the genes that derive MetS and to develop new therapies. Caveolin-1 (CAV-1) is a key protein component of caveolae and has been widely studied in dyslipidaemia and CVD due to signal transduction, trafficking in cholesterol haemostasis and TAG metabolism<sup>(7)</sup>. Mice treated with CAV-1 are resistant to high-fat diets, and they show lipodystrophy, hypertension, insulin resistance and abnormal glucose metabolism<sup>(8)</sup>. Moreover, several studies have indicated that CAV-1 genetic

variations might interact with other risk factors, including dietary intake of fatty acids, suggesting a positive association between CAV-1 and hypercholesterolaemia<sup>(9)</sup>. In studies conducted among Caucasian and Hispanic cohorts, the prevalence of CAV-1 gene variant rs926198 is related to higher odds of the MetS risk and low HDL<sup>(10,11)</sup>. Also, CAV-1 overexpression has been observed to relate to higher odds of atherosclerosis in experimental models<sup>(12)</sup>. The exact mechanisms are unclear, but it seems that CAV-1 is able to regulate several key enzymes in lipid metabolism, such as cholesterol ester transfer protein and phospholipid transfer protein<sup>(13)</sup>. While the association between CAV-1 polymorphisms and type 2 diabetes risk has been widely reported in various populations<sup>(14)</sup>, these relationships with the MetS have been inconsistent, despite several publications on the association between CAV-1 gene variants and serum lipid profiles<sup>(14–17)</sup>. To the authors' knowledge, there has been no study evaluating CAV-1 rs3807992 variant, metabolic risk factor and the interaction of fatty acid intake levels with this SNP. Hence, the aim of the current study was to evaluate the interaction of

**Abbreviations:** BP, blood pressure; CAV-1, caveolin-1; MetS, metabolic syndrome.

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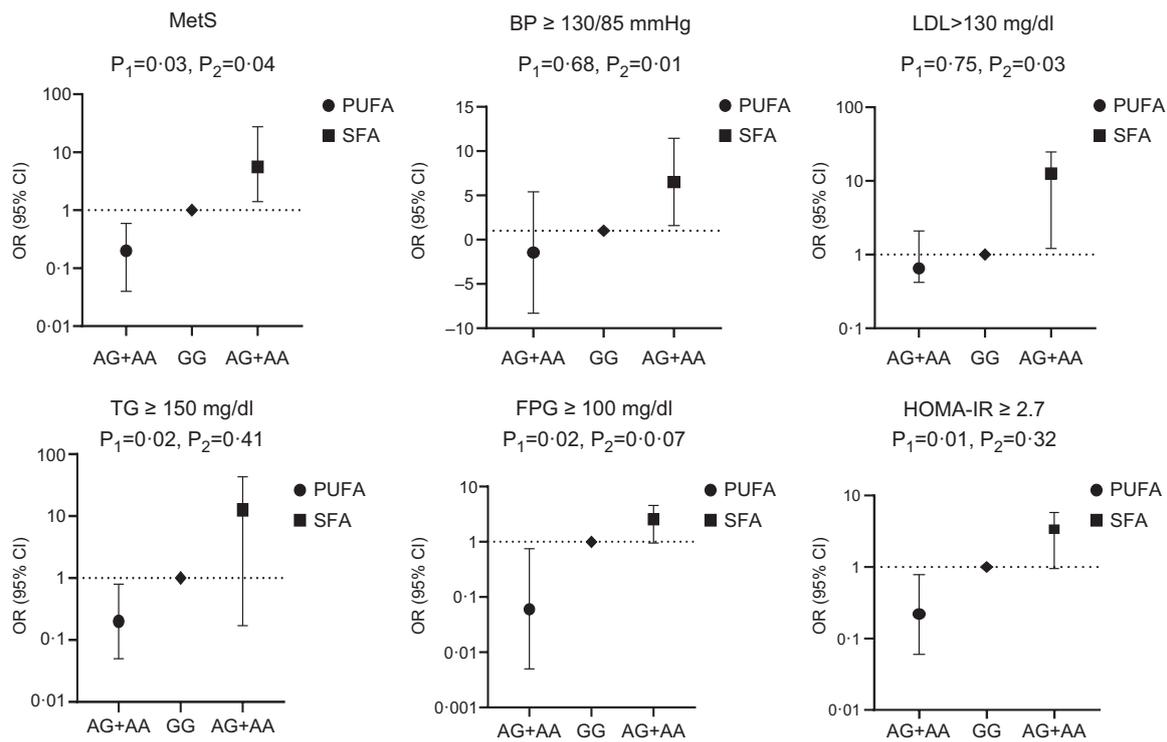


Fig. 1. Adjusted OR (95% CI) for the metabolic syndrome (MetS) and its components according to median of dietary fat intake and CAV-1 rs3807992

CAV-1 genetic polymorphism with the types of dietary fatty acids, in terms of the MetS risk factor status.

**Materials and methods**

*Study population*

For this cross-sectional study, 404 women in the range of 18–55 years old were recruited from health referral centres. Participants provided written informed consent. The inclusion criteria were: obese or overweight, no alcohol consumption and no smoking, CVD, hypertension, type 2 diabetes, polycystic ovary syndrome, kidney failure, stroke, thyroid disease, liver disease, cancer, inflammatory diseases and those who were on certain therapeutic drugs, weight loss programmes or supplements during the study time were all excluded. Each participant was interviewed in order to obtain demographic data and then referred to the laboratory for blood sampling. Anthropometric measures were taken, including: height (m), weight (kg), waist circumference (cm) measured at the narrowest part of the abdomen, and BMI (kg/m<sup>2</sup>, calculated by dividing weight by height squared). Blood pressure (BP) was measured with a sphygmomanometer (BP) after 5 min' rest. The study was approved by the Ethics Committee at the Tehran University of Medical Sciences (97-03-161-41017).

*The metabolic syndrome definition*

MetS cases were required to meet three or more of the following criteria according to the Adult Treatment Panel III criteria:

- 1) Elevated fasting blood glucose ≥ 100 mg/dl.

- 2) Hypertriacylglycerolaemia TAG ≥ 150 mg/dl
- 3) Elevated BP (≥ 130/85 mmHg)
- 4) Low (HDL-cholesterol) < 50 mg/dl in women
- 5) Waist circumference cut-off 80 cm (women) was considered as an indicator of abdominal obesity<sup>(18)</sup>.

*Dietary assessment*

The FFQ was a useful tool for evaluating dietary intake and included 147 items<sup>(19)</sup>. This assessment was carried out by interviewing the occurrence of food items consumed on the basis of a predetermined list of foods. The extracted FFQ values were then changed to g/d. For the evaluation of macro- and micronutrient content, N4 software was used; all measurements were then entered into IBM SPSS.

*Physical activity measurement*

Physical activity was measured using the International Physical Activity Questionnaire short form. Women's physical activity was divided into three categories: low (under 600), mild (600–3500) and extreme (over 3500) (MET-h/week). International Physical Activity Questionnaire's validity and reliability were tested in Iranian people<sup>(20)</sup>.

*Genotyping*

For genotyping the CAV-1 polymorphisms, DNA was extracted from whole blood via a Mini Columns kit (Type G; Genall; Exgene). The PCR-restriction fragment length polymorphism technique was employed to investigate

CAV-1 polymorphisms (rs3807992) in gene fragments (major allele G and minor allele A). PCR was carried out using the following primers: **F:**3'AGTATTGACCTGATTGCCATG5' **R:**5'GTCTTCTGAAAAAGCACATGA-3'. PCR were performed in a volume of 20 µl, containing 50 ng extracted DNA, 10 µM Forward primers, 10 µM, Reverse primers, 7 µl distilled water and Taq DNA Polymerase Master Mix (2X) in a DNA thermocycler. The DNA templates were denatured at 94°C for 3 min and 40 cycles, including a min denaturation at 94°C, a min annealing at 42–50°C and elongation at 72°C for 2 min. Amplified DNA was digested with HinIII (NlaIII) (2.5 U) restriction enzyme at 37°C overnight and then separated by electrophoresis on an agarose gel (2.5 %). Fragments concluding three genotypes of the CAV-1 rs3807992 variant were detected: uncut homozygous AA (213bp), cut heterozygous GA (3 bands: 118 & 95 & 213 bp) and cut homozygous GG (2 bands: 118 & 95 bp).

**Biochemical analysis**

All blood samples were drawn, after 12–14 h of fasting, at the nutrition laboratory of Tehran University of Medical Sciences. Blood samples were centrifuged for 10 min at 3000 rpm to extract serums, then aliquoted into 1 ml tubes and stored at 70°C before analysis. Auto-analyzer BT 1500 (Selectra 2; Vital Scientific) was used to test the samples. Fasting plasma glucose was determined using the Glucose Oxidase Phenol 4-Aminoantipyrine Peroxidase process. TAG kits (Pars Azmoon Inc.) were used to measure serum TAG by colorimetric method tests with Glycerol-3-phosphate oxidase Phenol 4-Aminoantipyrine Peroxidase. The cholesterol oxidase-Phenol 4-Aminoantipyrine Peroxidase was used to calculate total cholesterol, and the direct method and immunoinhibition were used to measure LDL and HDL. Pars Azmoon (Pars Azmoon Inc.) provided all of the kits.

**Statistical analysis**

Statistical analysis was performed using SPSS version 25 software (SPSS Inc.), and significance level was considered  $P < 0.05$ . The Kolmogorov–Smirnov was utilised to test the normality of the data, and all data were expressed as means and standard deviation. The Pearson’s  $\chi^2$  test was used to determine the Hardy–Weinberg Equilibrium deviation among allele frequency of CAV-1 G32124A (rs3807992). We used the dominant model for genetic analysis, risk allele carriers (AA, AG) compare with homozygotes major allele (GG). Medians and interquartile ranges were calculated for dietary intakes between CAV-1 genotypes. Mann–Whitney  $U$  test and Quade’s ANCOVA were used for associations between dietary and genotypes in crude and adjusted models, respectively. Independent-sample  $t$  test was used to evaluate the differences between the two groups. Binary logistic regression was used to estimate interactions between rs3807992 and high and low dietary fat intake on the OR of the MetS component. Interaction analyses were adjusted for variables proven to be related to MetS components (such as age, physical activity, energy intake, BMI, age at onset of obesity, and total SFA and PUFA intake).

**Table 1.** Clinical characteristics of all subjects based on caveolin-1 (CAV-1) rs3807992 genotypes (Mean values and standard deviations)

	(GG) (AG/AA)				P-value*
	Mean	SD	Mean	SD	
Age (years)	37.56	9.49	35.75	8.78	0.05
Height (cm)	161.30	6.08	160.96	5.58	0.58
Weight (kg)	79.71	10.91	82.12	13.23	0.07
BMI (kg/m <sup>2</sup> )	30.68	4.01	31.66	4.46	<b>0.02</b>
WC (cm)	98.22	9.30	100.48	10.38	<b>0.02</b>
FPG (mg/dl)	87.98	9.62	86.95	9.75	0.36
LDL-cholesterol (mg/dl)	98.80	22.66	91.27	25.07	<b>0.006</b>
HDL-cholesterol (mg/dl)	49.7	11.16	44.04	10.16	<b>&lt; 0.0001</b>
TC (mg/dl)	186.76	33.74	182.71	37.36	0.30
TAG (mg/dl)	113.11	51.20	133.31	84.14	0.13
SBP (mmHg)	109.6	15.05	112.90	14.75	0.08
DBP (mmHg)	75.87	10.77	79.31	10.06	<b>0.01</b>

WC, waist circumference; FPG, fasting plasma glucose; TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure. Comparisons between groups were determined based on independent-samples  $t$  test. Bold values indicate statistical significance ( $P < 0.05$ ).

**Results**

*Clinical characteristics according to caveolin-1 rs3807992 genotypes*

The overall prevalence of rs3807992 genotypes was 38.34% and 61.66 % for the A and G alleles. The genotype distributions had a deviation from HWE ( $P < 0.05$ ). Table 1 expresses the mean clinical characteristics of all women according to CAV-1 genotypes. It was observed that the (AA + AG) group had significantly higher BMI, waist circumference and DBP ( $P = 0.02$ ,  $P = 0.02$  and  $P = 0.01$ , respectively). Furthermore, women with (AA + AG) allele had significantly lower serum LDL ( $P = 0.006$ ) and HDL ( $P < 0.0001$ ) than the GG group. Additionally, no significant differences were detected for age, height, weight, FBG, TAG and SBP.

*Genotype frequencies between the metabolic syndrome and control groups*

Distributions of allele frequencies and the effect size of rs3807992 on various genetic models (codominant, dominant, recessive) had significant differences between the MetS and control groups. In the codominant model, the A homozygous allele for the CAV-1 (rs3807992) gene was 52 % higher in the control group than the MetS group (OR 2.52 (1.11, 5.70),  $P = 0.02$ ). In the dominant model, the A homozygous allele for the CAV-1 (rs3807992) gene was 31% higher in the control group than the MetS group (OR 2.31 (1.16, 4.61),  $P = 0.01$ ) (Table 2).

*Associations between caveolin-1 rs3807992 and the risk of the metabolic syndrome and its components*

It was found that (rs3807992) A allele carriers were at higher odds of the MetS (OR 2.31 (1.16, 4.16),  $P = 0.01$ ), abdominal obesity (OR 1.42 (0.98, 2.06),  $P = 0.06$ ), increased TAG concentration (OR 2.12 (1.13, 3.95),  $P = 0.01$ ), elevated BP (OR 7.03 (1.43, 34.44),  $P = 0.01$ ), increased glucose concentration (OR

**Table 2.** Caveolin-1 (*CAV-1*) rs3807992 frequencies between the metabolic syndrome (MetS) and control groups (Numbers and percentages)

Models	SNP rs3807992	Frequencies for the CAV-1 SNP				OR	95 %CI	P-value
		Control		MetS				
		n	%	n	%			
Codominant	GG	94	55.2 %	30	45.5 %			0.06
	AG	31	18.4 %	13	19.7 %	2.04	0.79, 5.26	0.13
	AA	41	24.7 %	23	34.8 %	2.52	1.11, 5.70	<b>0.02</b>
Dominant	GG	94	56.6 %	30	45.5 %			
	AG/AA	72	43.4 %	36	54.5 %	2.31	1.16, 4.61	<b>0.01</b>
Recessive	AG/GG	125	75.3 %	43	65.2 %			
	AA	41	24.7 %	23	34.8 %	2.04	0.97, 4.31	0.05

Comparisons between groups were determined based on logistic regression analysis. Bold values indicate statistical significance ( $P < 0.05$ ).

**Table 3.** Associations between caveolin-1 (*CAV-1*) rs3807992 and the risk of the metabolic syndrome (MetS) and its components (Odds ratios and 95 % confidence intervals)

	(AG/ AA) v. GG		P-value
	OR	95 %CI	
MetS	2.31	1.16, 4.61	<b>0.01</b>
Abdominal obesity $\geq 80$ cm	1.42	0.98, 2.06	0.06
BP $\geq 130/85$ mmHg	7.03	1.43, 34.44	<b>0.01</b>
FPG $\geq 100$ mg/dl	0.7	0.27, 1.78	0.45
HDL-cholesterol $< 50$ mg/dl	-1.4	1.02, 1.93	<b>0.03</b>
TAG $\geq 150$ mg/dl	2.12	1.13, 3.95	<b>0.01</b>

BP, blood pressure; FPG, fasting plasma glucose.

Comparisons between groups were determined based on binary logistic regression analysis adjusted by age.

Bold values indicate statistical significance ( $P < 0.05$ ).

0.7 (0.27, 1.78),  $P = 0.45$ ) and decreased HDL-cholesterol concentration (OR -1.4 (1.02, 1.93)  $P = 0.03$ ) (Table 3).

### Dietary intake relationships between caveolin-1 rs3807992 gene polymorphisms and their interaction on the metabolic syndrome and its components

The results of dietary nutritional intake are described according to genotype groups, which show no significant differences were found in nutrient consumption values (Table 4).

It was observed that the interaction of CAV-1 rs3807992 gene polymorphism and SFA intake was significant on the MetS (OR 5.60 (1.14, 27.40),  $P = 0.03$ ) and its components, including LDL (OR 12.95 (1.21, 24.69),  $P = 0.03$ ), BP (OR 6.52 (1.59, 11.45),  $P = 0.01$ ) in the multi-adjusted model (adjusted for age, energy intake, BMI, age at onset of obesity and total PUFA intake). There was no further significant interaction between the (AA + AG) group and SFA intake on other biochemical parameters, including HDL and TAG in both the crude and adjusted models ( $P > 0.05$ ) (Table 5; Fig. 1).

Interestingly, when CAV-1 (AA + AG) group interaction was analysed by PUFA intake, the relationship was significant in terms of the MetS (OR -0.207 (0.04, 0.95),  $P = 0.04$ ) and its components, including TAG (OR -0.27 (0.05, 0.8),  $P = 0.02$ ), fasting plasma glucose (OR -0.06 (0.005, 0.75),  $P = 0.02$ ) and homoeostasis model assessment insulin resistance (OR -0.22 (0.06, 0.78),  $P = 0.01$ ) in

the multi-adjusted model (adjusted for age, energy intake, BMI, age at onset of obesity and SFA intake) (Table 6; Fig. 1).

## Discussion

According to these findings, female A allele carriers had significantly higher BMI, waist circumference and DBP and had lower serum LDL and HDL, compared with GG genotypes. This demonstrates that their clinical parameters are predisposed to the MetS. A allele carriers were at higher odds of the MetS. To the authors' knowledge, there is little evidence describing an association between CAV-1 and the MetS. It was observed that a genetic variant of CAV-1 (rs3807992) was associated with increased MetS risk, which is consistent with previous studies that revealed a significant association between the minor allele in CAV-1 variations and the odds of metabolic diseases<sup>(10,11,15,17)</sup>. Previous studies by our research team have shown association between CAV-1 rs3807992 and metabolic disease<sup>(21,22)</sup>. Also, compared with other candidate gene studies, no studies had evaluated SNP rs3807992 and MetS risk.

It is proposed that CAV-1 polymorphisms increase MetS risk through altered CAV-1 gene expression, attenuating dyslipidaemia and hypertension, while impairing glucose and insulin homoeostasis<sup>(12-14,23)</sup>. CAV-1 regulates signalling molecules, such as IRS1, that have a key role in appropriate insulin responses, protein kinase A, angiotensin II receptors, active BP molecules, binding sites for Ca ions, insulin, lipids and hormone metabolisms; all of these may affect various clinical traits of the MetS<sup>(14,24-26)</sup>. Apart from genetic mutations, increasing evidence indicates that epigenetic modifications can provide an upstream regulatory switch to regulate the expression of CAV-1 thus leading to disease conditions<sup>(27)</sup>. Most of the recent research focuses on the effects of methylation on CAV1 expression. MiRNA-103 and 107 are up-regulated in obese mice, which can lead to impaired glucose homoeostasis, insulin receptor stabilisation and a receptive insulin signalling system<sup>(28,29)</sup>. In lipid metabolism, DHA has been shown to modify the transcriptome of miRNA. The expression of miRNA-192 in endothelial cells is greatly increased by DHA, and CAV1 is estimated to be a target for miRNA-192. CAV1 expression is decreased when miRNA-192 is overexpressed<sup>(30)</sup>.

**Table 4.** Dietary fat intakes of all subjects based on caveolin-1 (*CAV-1*) *rs3807992* genotypes (Median values and interquartile ranges (IQR))

	GG		AG/AA		P value*	P value†
	Median	IQR	Median	IQR		
Energy (kcal)	2490.89	1137.99	2567.98	1237.64	0.5	
Total Fat (g)	89.24	46.04	90.50	51.60	0.64	0.46
Protein (g)	88.23	41.65	87.59	43.77	0.73	0.79
Carbohydrate (g)	351.45	158	355.30	166	0.51	0.45
SFA (g)	25.59	14.61	26.38	14.44	0.91	0.45
Cholesterol (g)	245.47	146.83	237.49	140.89	0.23	0.42
Total fibre (g)	43.75	25.19	44.31	28.10	0.56	0.54
PUFA intake, %E	4.75	3.01	5.95	2.5	0.47	0.75
MUFA intake, %E	10.56	3.37	10.55	3.63	0.69	0.31
n-6 PUFA intake, %E	5.30	3	5.38	2.47	0.51	0.54
n-3 PUFA intake, %E	0.41	0.23	0.43	0.26	0.46	0.51

Mann–Whitney *U* test and Quade’s ANCOVA were performed to identify significant differences between *CAV-1 rs3807992* genotypes in crude and adjusted model respectively.

\* (P value): crude model.

† (P value): adjusted model by potential confounding factors (age, energy intake, educational level, DBP).

**Table 5.** Interactions between the caveolin-1 (*CAV-1*) *rs3807992* and SFA intake in relation to the metabolic syndrome (MetS) and its components (Odds ratios and 95 % confidence intervals)

	SFA (≥25 g/d)* <i>rs3807992</i>		
	OR (AG/AA) 1(Ref)	95 % CI	P value*
	GG		
MetS	5.60	1.14, 27.40	0.03
BP ≥ 130/85 mmHg	6.52	1.59, 11.45	0.01
HDL-cholesterol < 50 mg/dl	-0.87	-6.32, 4.58	0.75
LDL > 100 mg/dl	12.95	1.21, 24.69	0.03
TAG ≥ 150 mg/dl	12.64	-17.89, 43.19	0.41

BP, blood pressure.

Binary logistic was performed.

\* P-value: adjusted model by potential confounding factors (age, physical activity, energy intake, BMI, age at onset of obesity, and total PUFA intake).

**Table 6.** Interactions between the caveolin-1 (*CAV-1*) *rs3807992* and PUFA intake in relation to the metabolic syndrome (MetS) and its components (Odds ratios and 95 % confidence intervals)

	PUFA (≥ 6 % energy)* <i>rs3807992</i>		
	OR (AG/AA) 1(Ref)	95 % CI	P value*
	GG		
MetS	-0.2	0.04, 0.95	0.04
TAG ≥ 150 mg/dl	-0.2	0.05, 0.8	0.02
FPG ≥ 100 mg/dl	-0.06	0.005, 0.75	0.02
HOMA-IR ≥ 2.7	-0.22	0.06, 0.78	0.01

FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment insulin resistance.

Binary logistic was performed.

P-value\*: adjusted model by potential confounding factors (age, physical activity, energy intake, BMI, age at onset of obesity and total SFA intake).

Most previous intervention studies have shown that environmental factors, in particular dietary fat composition, may alter the risk of the MetS. Based on previous studies, higher intake of SFA was detrimental to maintaining insulin sensitivity, whereas PUFA showed beneficial effects<sup>(31)</sup>. Additionally, nutrigenetic research has indicated that dietary fat background can influence

genotype–phenotype relations<sup>(32)</sup>. In our study, the genetic association between *CAV-1* polymorphisms and the MetS by dietary fat intake was reported. High dietary SFA intake (≥25 g) especially accentuated the negative effects of *rs3807992* in terms of MetS risk.

The evidence for the *CAV1* gene and nutrient is limited so far, and it is mostly based on animal studies. In mice models, the role of dietary fat in the relationship between the *CAV1* gene and lipid and glucose metabolism has been investigated previously. *CAV-1* KO mice have demonstrated resistance to obesity diet, as well as increased plasma levels of chylomicron/VLDL particles and TAG caused by a defect in lipid droplet formation and hydrolysis<sup>(33)</sup>. Under fat diet conditions, *CAV-1* null mice have shown white adipose tissue atrophy and TAG deposition in plasma. Besides, *CAV-1* knockout mice fed a western-type diet represent a significant rise in VLDL and IDL/LDL<sup>(34)</sup>.

Besides, *CAV-1* has also been observed moving from the plasma membrane to lipid droplets in response to NEFA<sup>(35)</sup>. *CAV-1*’s function in lipid transport had also been confirmed by *CAV-1*-deficient mice’s lean phenotype, which had significantly higher serum levels of NEFA and TAG in the postprandial state<sup>(36–38)</sup>. Accordingly, caveolae are a main centre for several nutrient metabolisms through the cell membrane and play a unique function in the uptake of various lipid and glucose metabolites<sup>(39)</sup>. Caveolae are able to uptake fatty acid, TAG and cholesterol in many tissues, which leads to an elevation in caveolae density in obese rats<sup>(40,41)</sup>. In line with previous studies, experimental studies have reported that sphingomyelin is a key phospholipid of caveolae. SFA intake may lead to increases in sphingolipids levels in the cardiac cell membranes, thus disrupting the caveolae contents<sup>(42,43)</sup>. Furthermore, *CAV-1* mRNA levels are up-regulated by free cholesterol in human cells<sup>(44)</sup>. An appropriate level of cholesterol in the cell membrane under dietary fat conditions causes the caveolae activity are disrupted<sup>(45)</sup>. Experimental studies suggest that lipid abnormalities can influence caveolae formation and function, thereby disrupting fatty acid metabolism and contributing to the development of the MetS and obesity<sup>(46)</sup>.

A further novel finding is that high PUFA intake (≥6 % energy) reduced the negative effects of *rs3807992* in terms of MetS risk, with

the greatest protection achieved by A allele carriers. Caveolae membrane fatty acids (in the internal and external leaflet) are also significantly altered by *n*-3 PUFA intake and are even able to change the function of the caveolae. In this regard, Chapkin *et al.* reported that in animal models, *n*-3 PUFA intake may modulate the function of caveolae proteins/lipid, affecting membrane fusion and cell–cell signalling, and improving insulin signalling<sup>(47)</sup>. Previous studies have indicated that H-Ras and endothelial nitric-oxide synthase are moved from caveolae in *n*-3 PUFA-supplemented rats, which suppressed the Ras-dependent signalling and reduced BP, thereby lowering the MetS risk<sup>(48,49)</sup>. CAV-1 prevents the production of nitric oxide. These findings demonstrate that PUFA intake affects CAV-1 abundance, which in turn affects endothelial nitric-oxide synthase activity and, as a result, vascular function<sup>(50,51)</sup>.

Besides, CAV-1 can regulate fatty acid translocase surface accessibility by acting as an intracellular shuttle for long-chain fatty acids to lipid droplets and thus indirectly control long fatty acid uptake<sup>(33,37,38)</sup>. Fatty acid translocase is required for long-chain fatty acid uptake. CAV-1 deficiency has previously been linked to a total loss of caveolae, the lack of fatty acid translocase cellular membrane expression and a decrease in fatty acid uptake<sup>(52)</sup>.

In this research, we observed that a PUFA can be effective on glucose profile in risk allele carriers. Changes in insulin receptor activity are one of the proposed pathways for the effect of dietary fat and CAV-1 on metabolic markers. According to strong evidence, caveolae are implicated in the pathogenesis of insulin receptor and the MetS<sup>(14)</sup>. If animals were fed a high-fat diet, insulin receptor substrate 1 and Protein Kinase B showed reduced activity, indicating insulin resistance. These results point to a mechanism by which a high-cholesterol diet affected CAV-1 expression *in vivo*, as well as insulin receptor localisation and function<sup>(14,53,54)</sup>. It is suggested that a correlation between hyperlipidaemia and insulin receptor through caveolae under dietary fat conditions.

However, another mechanism by which fatty acids can alter the genetic risk posed by CAV-1 polymorphisms may also contribute to the apoA1<sup>(13)</sup>. Consistent with our results, CAV-1-deficient animals fed a western-style diet have significantly lower apoA-I plasma levels. Loss of CAV-1 contributes to the development of the MetS, according to apoA-I levels, the key protein marker of HDL<sup>(55)</sup>.

The mechanism observed in the present study, by which fatty acids are able to modify the genetic risk posed by CAV-1 polymorphisms, remains unknown; further studies are needed to indicate such gene–diet interventions. Caveolae and its components may become useful sites for further investigation into treating the MetS.

### Limitations

Several limitations can also be identified in the current study. Dietary intake was assessed by a FFQ, which is self-reported and thus dependent on patient memory. Due to financial limitations, it was not possible to perform western blot analysis to determine whether rs-3807992 SNP alters the expression of CAV-1. The focus of the current study was on dietary fat composition, but other nutrient components, including carbohydrates

or fibre, can also play a role in the progression of the MetS. Furthermore, lipid parameter measurements were taken, while the subjects were fasted, which may have obscured differences in CAV-1 under fed conditions. Given the observational nature of the study, it is not possible to tell whether the associations which were identified in women (but not men) are of a causal nature. Finally, we did not include a normal-weight participant due to financial constraints and instead focused on overweight and obese women as high-risk categories for the MetS<sup>(56)</sup>. However, as the first study in this regard, we recommend that future research concentrate on normal-weight people both men and women.

### Conclusion

To the authors' knowledge, this is the first study presenting the association of a genetic variant of CAV-1 rs3807992 with the risk of the MetS and its components, including TAG, BP and HDL level. However, further studies are needed to determine the strength of this association in a larger population; the contribution of this study is the novel finding that rs3807992 clearly predicts the MetS among obese women. Analyses of the individual components of the MetS confirmed that the rs3807992 variant is related to elevated BP, dyslipidaemia, low HDL-cholesterol and high TAG levels. Also, CAV-1 rs3807992 genotypes are sensitive to dietary SFA and PUFA, which allows individuals to monitor and adjust SFA and PUFA consumption accordingly. Finally, these results can be used in combination with a patient's genetic history in order to provide more applicable and tailored nutritional advice for preventing or attenuating the MetS in overweight and obese women.

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F. A. contributed to conception, design, data analyses, data interpretation and manuscript drafting. K. H. M. supervised the study. All authors approved the final manuscript for submission.

The authors declare no conflict of interest.

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