Molecular Biology Reports
https://doi.org/10.1007/s11033-019-04956-8

ORIGINAL ARTICLE

The influence of the fatty acid amide hydrolase 385C>A single nucleotide polymorphisms on obesity susceptibility

Neda Lotfi Yagin[1] · Fereshteh Aliasgari[1] · Soghra Aliasgharzadeh[1] · Reza Mahdavi[2] · Maryam Akbarzadeh[3,4]

Received: 2 April 2019 / Accepted: 27 June 2019

Abstract

The chronic over-activation of the endogenously produced cannabinoids in obesity has been demonstrated in several studies. A common 385C>A single nucleotide polymorphism of the fatty acid amide hydrolase, one the most important inactivating enzymes of endogenous cannabinoids, has been shown to be associated with obese phenotype. This study was designed to investigate the FAAH gene polymorphisms and to compare the obesity indices between different genotypes in Iranian overweight/obese women. A total of 180 healthy overweight/obese subjects (BMI = 25 to 40 kg/m^2) and 86 normal weight individuals (BMI = 18.5 to 24.9 kg/m^2) were genotyped for 385 C/A polymorphism of FAAH using amplification refractory mutation system (ARMS)-PCR. Anthropometric indices including BMI, waist circumference, neck circumference, waist to height ratio, fat mass were evaluated. A written informed consent form was given by the participants. The genotype and allele frequencies were significantly different between the overweight/obese and control groups (P = 0.04). Significant differences were observed between the CC genotype and the AA+CA genotype regarding the anthropometric indices (P < 0.05). Compared to CC group, a higher BMI, WC, WHtR, NC and fat mass was identified in allele A carriers group. After adjusting for age, marital and physical activity status, it was revealed that having the CA/AA genotype increased the probability of obesity risk almost two times (P < 0.05, 95% CI 1.19–3.67). Our findings showed that the frequency of A allele was greater in overweight/obese individuals. Also, a mutation in FAAH gene was associated with higher anthropometric indices and the CA/AA genotype increased significantly the possibility of being obese in Iranian women.

Keywords Obesity · Endocannabinoids · Polymorphism · Fatty acid amide hydrolase

Introduction

Obesity has become one of the most important health challenges in the last decades and its prevalence is worsening throughout the world [1]. It is projected that if secular trends continue, approximately 38% of the world's adult population will be overweight and 20% will be obese by 2030 [2]. Comparable to the developed countries, in developing countries the obesity rate has also tripled during the past years [3]. Iran is also one of the developing countries in which the obesity rate has elevated at an alarming speed and approximately 23.3% of the Iranian adults are obese [4]. Obesity provokes or exacerbates a number of pathologies alone or in conjunction with other health issues [5, 6]. Obesity mainly accounts for the incidence of several chronic diseases including cardiovascular disease, type two diabetes, and different types of cancers [7–9].

In addition to the role of environmental factors in overweight and obesity spread, previous reports has repeatedly

Reza Mahdavi and Maryam Akbarzadeh have contributed equally to this manuscript.

✉ Reza Mahdavi
mahdavir@tbzmed.ac.ir; mahdavirez@hotmail.com

✉ Maryam Akbarzadeh
maryamakbarzadehbio@gmail.com

[1] School of Nutrition and Food Sciences, Nutrition Research Center, Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

[2] School of Nutrition and Food Sciences, Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[3] Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Science, Tabriz, Iran

[4] Department of Biochemistry, Erasmus University Medical Center, Rotterdam, The Netherlands

Published online: 08 July 2019

emphasized that genetic factors play significant role in several adiposity traits, such as BMI, waist circumference, visceral and subcutaneous adipose tissue, and etc. [10–12]. It is believed that genetic factors and polymorphisms in related genes are currently responsible for 40–70% of the variance in human adiposity [13]. In the last few decades, the genes and molecules which are responsible for energy intake regulation have been spotted and studied widely [14–16].

Endogenous cannabinoids are lipid molecules produced from phospholipids or triglycerides of the membrane with several effects on metabolic regulation and body weight [17]. Different enzymes get involved in the synthesis and degradation of the two most significant endocannabinoids, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) [18]. Fatty acid amide hydrolase (FAAH) has been known as the key catabolic enzyme, capable of deactivating most of the endocannabinoids including AEA [19]. FAAH is a serine hydrolase and a missense c.385C>A single-nucleotide polymorphism (SNP) (rs324420) in the human FAAH gene replaces a conserved proline residue at amino acid position 129 to threonine [20]. This replacement can lead to a decrease in FAAH expression or activity, resulting in continued stimulation of the cannabinoid 1 receptor through the increased levels of the endogenous fatty acid amides which in turn increase appetite, food intake and body fat accumulation [21].

Although genetic variation in FAAH particularly the homozygous FAAH 385 A/A genotype has formerly been shown to be associated with overweight and obesity [22–24], others have failed to find such association [25, 26]. Additionally, there is scarcity of studies about the relation of FAAH gene polymorphisms with obese phenotypes in the Asian population. To this end, since determining of the common genetic variants that affect obesity risk at a population level might improve the pathophysiologic conception of obesity, this study was designed to evaluate the FAAH gene polymorphisms status and to compare the obesity indices between different genotypes in Iranian overweight/obese women.

Subjects and methods

Participants and study design

This cross-sectional study was carried out between October 2017 and January 2018. The study protocol was approved by local ethical committee (IR.TBZMED.REC.1396.620) and all the participants consented to use their genetic material and other necessary information. A total of 180 healthy overweight/obese subjects (BMI = 25 to 40 kg/m^2) and 86 normal weight individuals (BMI = 18.5 to 24.9 kg/m^2) were enrolled in this study and all the participants were collected from the same geographical area. Since the obesity rate and food craving is more prevalent among women, we carried out this research only among women. Those having history of any metabolic disorders such as diabetes, kidney and liver disease as well as pregnant and lactating women were excluded. The demographic and physical activity information of the participants was recorded using the appropriate questionnaire by trained dietitian. The individuals' physical activity was evaluated using the International Physical Activity Questionnaire (IPAQ).

Anthropometric and body composition

The measurement of anthropometric indices including weight, height, waist circumference (WC), neck circumference (NC), and blood pressure were carried out in line with standard protocols in fasting state [27, 28]. By dividing WC by height the waist-to-height ratio (WHtR) was determined. BMI was calculated as weight (kg) divided by the square of the height (m^2). Fat mass was evaluated by bioelectrical impedance analysis (BC-418MA, Tanita, Japan).

DNA extraction and genotyping

Fasting venous blood samples were collected for genotyping process. The genomic DNA extraction from peripheral blood cell was carried out using DNA extraction and purification kit (Qiagen, Cat No./ID: 51306) as manufacturer's protocol. Optical density measurement (quantitative method based) was used to determine the genomic DNA concentration. By calculating the absorbance ratio at 260 nm to absorbance at 280 nm (A260/A280) the samples purity was confirmed. The genotyping was carried out by the amplification refractory mutation system (ARMS)-PCR in which sequence-specific PCR primers allows amplification of test DNA only when the target allele is within the sample. The forward common primer 5′-GCCAGAGACAGCCAGGATGAGG-3′ and reverse primer 5′-ATAGAGCAGGCCCTGCCTTGG-3′ were used to amplify a 314 bp product to detect the wild types. Also, the forward common primer 5′-GCCAGAGACAGCCAGGATGAGG-3′ together with reverse primer 5′-ATAGAGCAGGCCCTGCCTTGT-3′ were used to amplify and detect 314 bp mutant forms. Moreover, to confirm the accuracy of PCR results and to spot any negative results on agarose gel Hemoglobin (Hb) primers with the following sequences were used as internal controls (110 bp). Hb Forward: 5′-ACACAACTGTGTTCACTAGC-3′, Hb Reverse: 5′-CAACTTCATCCACGTTCACC-3′ Fig. 1. The PCR reaction was performed in a total reaction volume of 30 µl as previously described [11]. The optimization was carried out and thermo cycler conditions were as follows: the initial denaturation at 96 °C for 5 min followed by 35 cycles of amplification, each cycle consisting of 30 s

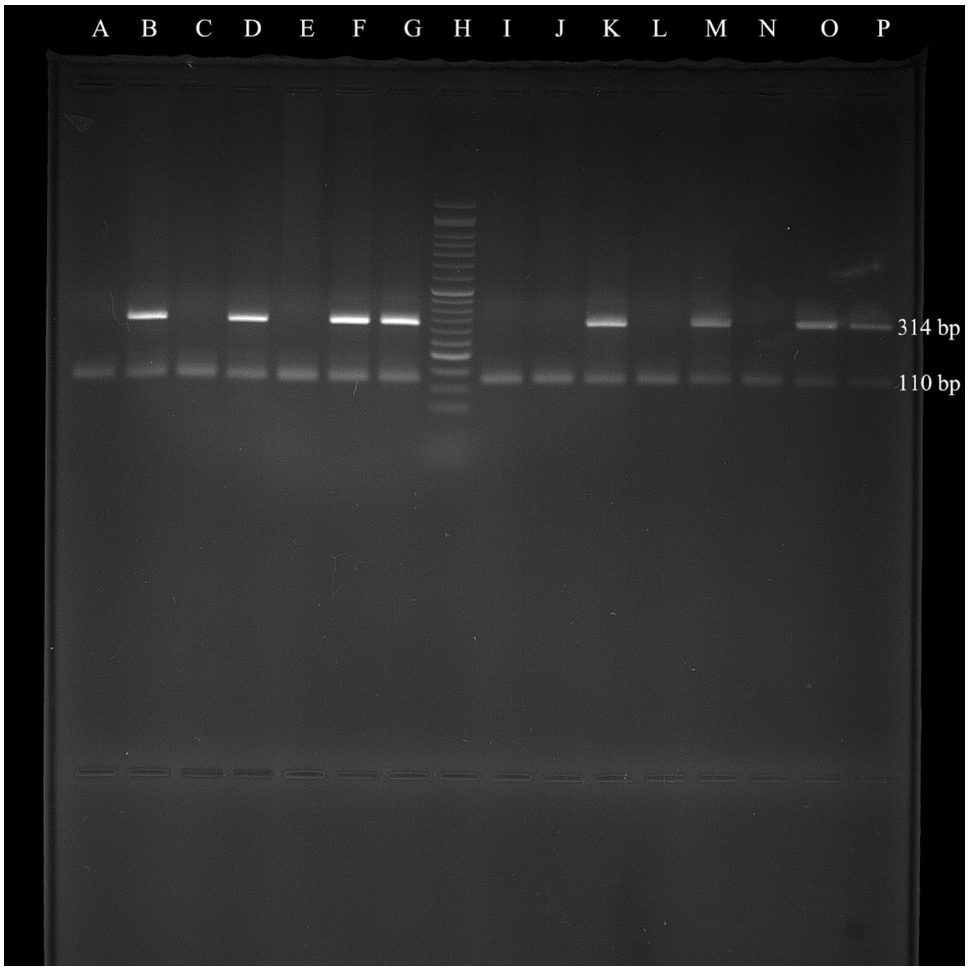

Fig. 1 Agarose gel (1%) electrophoresis for PCR products of FAAH C-385 A: Lane F,G; heterozygote C/A genotype, Lane B,C; homozygote C/C genotype, Lane D,E; homozygote A/A genotype, Lane H; 50 bp ladder, Lane O,P; positive control for C/A genotype, Lane K,L; positive control for homozygote C/C genotype, Lane M,N; positive control for homozygote A/A genotype, Lane A,I,J; negative control. 314 bp band; indicating C allele, 314 bp band; indicating A allele, 110 bp band; indicating internal positive control

at 96 °C, 62 s at 30 °C and 45 s at 72 °C, in in a peqlab PCR system. The reaction completed with a one more 7 min of extension at 72 °C. PCR products were electrophoresed on 1% agarose gel containing 5 µl/dl safe stain (Cinna Gen Co., Iran). Hardy–Weinberg equilibrium was evaluated.

Statistical analysis

Data were analyzed using SPSS software, version 21.0 (IBM Corp., Armonk, NY, USA). Normality of the data distribution was checked by the Kolmogorov–Smirnov goodness-of-fit test. Values are presented as mean ± SD for variables with normal distribution or median (25th; 75th percentiles) for variables without normal distribution. The chi- square test and Fisher's exact test were used to compare the FAAH 385 C/A genotypes between overweight/obese group as group 1 and control group as group 2. Two-tailed Student's *t* test and Mann–Whitney U-test was used to analyze the quantitative variables with normal distribution and nonparametric variables respectively. To predict the association of SNP with the risk of obesity logistic regression model was performed. In all statistical analyses except for Fisher's exact test, the C358A together with A358A were considered as a first group and wild type C358C as second group. To test the deviation of the observed genotype frequencies from the Hardy–Weinberg equilibrium (HWE), the Chi squared test was used in the overweight/obese and control samples. P value < 0.05 was regarded as statistically significant.

Results

The participants' demographic, anthropometric, and laboratory data are presented in Table 1. The study included 180 overweight/obese (mean age 34.2 ± 8.27 years old; 133 obese, 47 overweight) and 86 normal weight healthy women (mean age 33.1 years old ± 7.03). To test whether FAAH 385 C/A genotypes frequencies differ between overweight/obese and control group chi-square test and Fisher's exact test were used. As presented in Table 2 the genotype and allele frequencies were significantly different between the overweight/obese and control groups (P = 0.04, fisher exact test). Compared to normal weight women, a greater distribution of the CA (n = 70) and AA (n = 6) genotypes and a lower

Table 1 Participants' demographic, anthropometric, and laboratory data

	Control n = (86)	Overweight/obese n = (180)	P-value
Age	33.19 ± 7.03	34.2 ± 8.27	> 0.05*
Weight (kg)	57.97 ± 6.53	83.41 ± 11.31	< 0.05*
Height (cm)	161.43 ± 5.80	159.95 ± 5.59	> 0.05*
BMI (kg/m^2)	22.23 ± 2.08	32.54 ± 3.73	< 0.05*
WC (cm)	75 (69.75; 80)	101(95.25; 107)	< 0.05**
NC (cm)	32 (30; 33)	38 (36; 40)	< 0.05**
WHtR (cm)	0.46 (0.43; 0.49)	0.63 (0.59; 0.66)	< 0.05**
Fat mass (kg)	16.28 ± 5.02	34.35 ± 7.68	< 0.05*
SBP (mmHg)	107 (98; 110)	113 (103.25; 122.75)	< 0.05**
DBP (mmHg)	75 (69.75; 90)	77 (70; 82)	> 0.05**
TC (mg/dl)	161.45 ± 33.82	171.68 ± 30.75	< 0.05*
LDL (mg/dl)	81.87 ± 28.14	97.41 ± 25.31	< 0.05*
HDL (mg/dl)	61.29 ± 9.41	49.93 ± 9.03	< 0.05*
TG (mg/dl)	82.5 (69; 101)	103.5 (78; 151.25)	< 0.05**
FBS (mg/dl)	82 (76; 86)	89 (84; 99)	< 0.05**
Insulin (IU/ml)	6 (5; 9)	25.2 (16.3; 34)	< 0.05**
HOMA-IR	1.24 (0.98; 1.80)	5.56 (3.54; 7.99)	< 0.05**

Values are presented as mean ± SD for variables with normal distribution or median (25th; 75th percentiles) for variables without normal distribution

BMI body mass index, *WC* waist circumference, *NC* neck circumference, *WHtR* waist-to height ratio, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *TC* total cholesterol, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *TG* triglycerides (mg/dl), *FBS* fasting blood sugar

*Independent *t* test P value for differences between variables in control and overweight/obese group

**Mann–Whitney U test P value for differences between variables in control and overweight/obese group

distribution of the CC (n = 104) genotype was observed in overweight/obese group. Significant differences were observed between the homozygous genotype AA together with CA and wild homozygous CC regarding the anthropometric indices including BMI, WC, WHtR, NC and fat mass (P < 0.05) (Table 3). In comparison to CC group, a higher BMI, WC, WHtR, NC and fat mass was identified in allele A careers group for the polymorphism 385C>A in FAAH. With respect to the logestic regression analysis, after adjusting for age, marital and physical activity status it was revealed that having the CA/AA genotype increased the probability obesity risk almost two times (P < 0.05, OR 2.28 95% CI 1.19–3.67) (Table 4). In overweight/obese group, the 54.4% of the individuals had low levels of activity, 39.4% were moderately active and the rest of them were highly active (6.1%). Similarly, in normal weight participants, the 94.2% of the subjects had low levels of activity, 3.5% and 2.3% of them had moderate and high levels of activity respectively. The distribution of the FAAH cDNA 385C to A genotypes in both overweight/obese women (P > 0.05) and healthy controls (P > 0.05) did not deviate from HWE.

Discussion

The chronic over-activation of the endogenously produced cannabinoids in obesity has been demonstrated in several studies [29–31]. A common 385C>A single nucleotide polymorphism (SNP) of the FAAH, one the most important degrading enzymes of endocannabinoids, leads to a missense mutation making a FAAH with defective expression [32].

Present study indicated that the minor 385A allele was more usual in the overweight/obese patients compared to normal weight women. In overweight/obese individuals, the AA genotype percentage (3.3%) was comparable with other studies, for instance; 3.7% [22], 2.3% [26], and 1% [24]. Also, the CA genotype percentage (38.89%) was almost identical with other papers; 24.1% [22], 28.1% [26], and 36.5% [24]. The A allele of FAAH cDNA 385C to A SNP was also associated with a significant risk to develop obesity (OR 2.28, 95% CI 1.19–3.67). Moreover, BMI, WC, WHtR, NC and fat mass was higher in CA+AA group compared to individuals with CC genotypes.

The association of FAAH gene polymorphism and mutant allele carriers with obese phenotypes has been investigated in previous studies which yield contradictory results. In a study by Chiang et al. in 2004, it was reported that FAAH enzymatic activity and protein expression in FAAH 385 A/A missense polymorphism individuals was almost half of the wild-type subjects [33]. Also, in Caucasian women cDNA 385 C to A missense polymorphism in the FAAH was associated with overweight/obesity but not with binge eating disorder according to Monteleone et al. [24]. Furthermore, poorer cardiovascular status (BMI, weight, WC, TNF-alpha, insulin, and adiponectin levels) has been found in obese

Table 2 Genotype and allele frequencies of the 385 C/A polymorphism of FAAH between overweight/obese subjects and control individuals

Group	n	Genotype			Allele	
		CC	CA	AA	C	A
Overweight/obese	180	104 (57.78%)	70 (38.89%)	6 (3.33%)	77.22%	22.77%
Control	86	63 (73.26%)	22 (25.58%)	1 (1.16%)	86.05%	13.95%

Fisher's exact test value = 5.99, P-value = 0.04

Table 3 Anthropometric and clinical findings in participants according to 385 C/A polymorphism of FAAH gene

Characteristics	CC n = 167	CA + AA n = 99	P-value
BMI (kg/m^2)	27.75 ± 5.17	31.66 ± 6.10	< 0.05*
WC (cm)	92 (79; 100)	103 (84; 110)	< 0.05**
NC (cm)	36 (32; 38)	37 (33; 40)	< 0.05**
WHtR (cm)	0.57 (0.48; 0.62)	0.63 (0.53; 0.68)	< 0.05**
Fat mass (kg)	25.84 ± 9.63	33.01 ± 11.56	< 0.05*
SBP (mmHg)	110 (101; 117)	111 (104; 122)	< 0.05**
DBP (mmHg)	76 (69; 83)	77 (72; 85)	0.166**

Values are presented as mean ± SD for variables with normal distribution or median (25th; 75th percentiles) for variables without normal distribution. Abbreviations—see Table 1

*Independent *t* test P value for differences between variables in CC group and CA+AA subjects

**Mann–Whitney U test P value for differences between variables in CC group and CA+AA subjects

Table 4 The prediction power weight status by CA+AA genotype based on logistic regression analysis

	OR	SE	Sig.	95% CI	
				Lower	Upper
Genotype*					
CA + AA	2.28	0.7	0.008	1.37	3.79

Reference group was those with CC genotype

*Adjusted for age, marital and physical activity status

minor A allele carrier [34]. However, in a 5801 of Danish population, no association existed between the FAAH A allele and BMI, WHR, WC, HOMA-IR [25]. Interestingly, there were other studies which revealed that the A allele has been associated with lower blood pressure, with improved cardiovascular profile and larger reduction in glucose, LDL, BMI, WC, triglycerides and total cholesterol under low fat and hypocaloric diet [35].

According to the evidence, half of the FAAH enzymatic activity and protein expression has been seen in minor A allele carriers in comparison to wild-type subjects leading to considerably elevated levels of AEA [36]. In a study by Sipe et al. carried out on 48 normal weight subjects (BMI of ≤ 26 kg/m^2) and 96 severe obese individuals (BMI of ≥ 40 kg/m^2) significant elevation of AEA levels and similar substances in carriers of the FAAH 385 A mutant alleles were identified [22]. In another study, Martins et al. reported a remarkable association between increased AEA levels and the AA homozygous genotype in a multiethnic Brazilian population with a broad range of adiposity values [37]. We also noticed that AEA and 2-AG levels correlated positively with obesity indices including BMI, WC and BF% (data are not shown). Likewise, compared with their wild-type littermates, FAAH–/– mice exhibited increased energy storage [38]. AEA is one of the main endocannabinoids which can stimulate the CB1 receptor and has the capability of arousing appetite and feeding behavior which ultimately might promote the diet-induced obesity [39]. Additionally, the FAAH A385A could trigger the up regulation of natural reward behaviors such as palatable and sweets food consumption related to overweight and obesity. Indeed, the anatomical intersection of CB1 receptors and FAAH enzyme supports the view that FAAH is placed for endocannabinoid levels regulation that could affect craving and reward behaviors over the pertinent neuronal circuitry and eventually the promotion of energy storage [40].

However, the discrepancies seen in different studies regarding the association of FAAH gene polymorphism with obese phenotype might stem from the fact that further gene–gene or gene–environment interactions are responsible in the obesity appearance and metabolic abnormalities and polymorphisms are not the sole contributing factors.

In summary, the frequency of A allele was greater in overweight/obese individual and mutation in FAAH gene was associated with higher anthropometric indices in Iranian women. This study provides additional support for peripheral endocannabinoid system role in obesity and associated comorbidities which could possibly help to detect the subjects with a genetic susceptibility to obesity whom might gain advantage from primary prevention approaches. However, to improve the precision of our study, larger samples and further information about the metabolic and environmental factors which control the FAAH gene expression is required.

Acknowledgements The authors wish to thank the participants for their cooperation, time and patience and Tabriz University of Medical Sciences for the financial support. The results of this paper are from Neda Lotfi's Ph.D. thesis. The authors declare that there is no conflict of interest.

References

1. Aliasghari F, Yaghin NL, Mahdavi R (2019) Relationship between hedonic hunger and serum levels of insulin, leptin and BDNF in the Iranian population. Physiol Behav 199:84–87. https://doi.org/10.1016/j.physbeh.2018.11.013

2. WHO (2018) Obesity and overweight. http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed 18 Feb 2018
3. Hossain P, Kawar B, El Nahas M (2007) Obesity and diabetes in the developing world—a growing challenge. N Engl J Med 356(3):213–215. https://doi.org/10.1056/NEJMp068177
4. Barzin M, Valizadeh M, Serahati S, Mahdavi M, Azizi F, Hosseinpanah F (2018) Overweight and obesity: findings from 20 years of the tehran lipid and glucose study. Int J Endocrinol Metab 16(4 Suppl):e84778. https://doi.org/10.5812/ijem.84778
5. Ebrahimzadeh Attari V, Malek Mahdavi A, Javadivala Z, Mahluji S, Zununi Vahed S, Ostadrahimi A (2018) A systematic review of the anti-obesity and weight lowering effect of ginger (*Zingiber officinale* Roscoe) and its mechanisms of action. Phytother Res 32(4):577–585
6. Attari VE, Mahluji S, Jafarabadi MA, Ostadrahimi A (2015) Effects of supplementation with ginger (*Zingiber officinale* Roscoe) on serum glucose, lipid profile and oxidative stress in obese women: a randomized, placebo-controlled clinical trial. J Pharm Sci 21(4):184–191
7. Eckel RH, Alberti K, Grundy SM, Zimmet PZ (2010) The metabolic syndrome. Lancet 375(9710):181–183
8. Simmons R, Alberti K, Gale E, Colagiuri S, Tuomilehto J, Qiao Q, Ramachandran A, Tajima N, Mirchov IB, Ben-Nakhi A (2010) The metabolic syndrome: useful concept or clinical tool? Report of a WHO expert consultation. Diabetologia 53(4):600–605
9. Mathieu P, Poirier P, Pibarot P, Lemieux I, Després J-P (2009) Visceral obesity: the link among inflammation, hypertension, and cardiovascular disease. Hypertension 53(4):577–584
10. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR et al (2015) Genetic studies of body mass index yield new insights for obesity biology. Nature 518:197. https://doi.org/10.1038/nature14177
11. Akbarzadeh M, Hassanzadeh T, Saidijam M, Esmaeili R, Borzouei S, Hajilooi M, Mahjub H, Paoli M (2012) Cholesteryl ester transfer protein (CETP) – 629C/A polymorphism and it, s effects on the serum lipid levels in metabolic syndrome patients. Mol Biol Rep 39(10):9529–9534
12. Maroufi NF, Farzaneh K, Alibabrdel M, Zarei L, Cheraghi O, Soltani S, Montazersaheb S, Akbarzadeh M, Nouri M (2016) Taq1B polymorphism of cholesteryl ester transfer protein (CETP) and its effects on the serum lipid levels in metabolic syndrome patients. Biochem Genet 54(6):894–902. https://doi.org/10.1007/s10528-016-9766-5
13. Herrera BM, Keildson S, Lindgren CM (2011) Genetics and epigenetics of obesity. Maturitas 69(1):41–49
14. Hess ME, Brüning JC (2014) The fat mass and obesity-associated (FTO) gene: obesity and beyond? Biochim et Biophys Acta (BBA)—Mol Basis Dis 1842(10):2039–2047. https://doi.org/10.1016/j.bbadis.2014.01.017
15. Loos RJ (2012) Genetic determinants of common obesity and their value in prediction. Best Pract Res Clin Endocrinol Metab 26(2):211–226
16. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C (2009) Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41(1):25
17. Bermudez-Silva FJ, Cardinal P, Cota D (2012) The role of the endocannabinoid system in the neuroendocrine regulation of energy balance. J Psychopharmacol (Oxford, England) 26(1):114–124. https://doi.org/10.1177/0269881111408458
18. Martins C, Genelhu V, Di Marzo V, Francischetti E (2014) The endocannabinoid system–back to the scene of cardiometabolic risk factors control? Horm Metab Res 46(08):529–536
19. Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384(6604):83
20. Cable JC, Tan GD, Alexander SP, O'Sullivan SE (2011) The activity of the endocannabinoid metabolising enzyme fatty acid amide hydrolase in subcutaneous adipocytes correlates with BMI in metabolically healthy humans. Lipids Health Dis 10(1):129
21. Harismendy O, Bansal V, Bhatia G, Nakano M, Scott M, Wang X, Dib C, Turlotte E, Sipe JC, Murray SS (2010) Population sequencing of two endocannabinoid metabolic genes identifies rare and common regulatory variants associated with extreme obesity and metabolite level. Genome Biol 11(11):R118
22. Sipe J, Waalen J, Gerber A, Beutler E (2005) Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). Int J Obes 29(7):755
23. Müller TD, Brönner G, Wandolski M, Carrie J, Nguyen TT, Greene BH, Scherag A, Grallert H, Vogel CI, Scherag S (2010) Mutation screen and association studies for the fatty acid amide hydrolase (FAAH) gene and early onset and adult obesity. BMC Med Genet 11(1):2
24. Monteleone P, Tortorella A, Martiadis V, Di Filippo C, Canestrelli B, Maj M (2008) The cDNA 385C to A missense polymorphism of the endocannabinoid degrading enzyme fatty acid amide hydrolase (FAAH) is associated with overweight/obesity but not with binge eating disorder in overweight/obese women. Psychoneuroendocrinology 33(4):546–550
25. Jensen DP, Andreasen CH, Andersen MK, Hansen L, Eiberg H, Borch-Johnsen K, Jørgensen T, Hansen T, Pedersen O (2007) The functional Pro129Thr variant of the FAAH gene is not associated with various fat accumulation phenotypes in a population-based cohort of 5,801 whites. J Mol Med 85(5):445–449
26. Papazoglou D, Panagopoulos I, Papanas N, Gioka T, Papadopoulos T, Papathanasiou P, Kaitozis O, Papatheodorou K, Maltezos E (2008) The fatty acid amide hydrolase (FAAH) Pro129Thr polymorphism is not associated with severe obesity in Greek subjects. Horm Metab Res 40(12):907–910
27. Grundy SM, Neeland IJ, Turer AT, Vega GL (2013) Waist circumference as measure of abdominal fat compartments. J Obes 2013:9. https://doi.org/10.1155/2013/454285
28. Lin S, Hu L, Li P, Li X, Lin K, Zhu B, Mu P, Zeng L (2018) Utility of neck circumference for identifying metabolic syndrome by different definitions in chinese subjects over 50 years old: a community-based study. J Diabetes Res 2018:8. https://doi.org/10.1155/2018/3708939
29. Di Marzo V (2008) The endocannabinoid system in obesity and type 2 diabetes. Diabetologia 51(8):1356–1367. https://doi.org/10.1007/s00125-008-1048-2
30. Engeli S, Böhnke J, Feldpausch M, Gorzelniak K, Janke J, Bátkai S, Pacher P, Harvey-White J, Luft FC, Sharma AM (2005) Activation of the peripheral endocannabinoid system in human obesity. Diabetes 54(10):2838–2843
31. Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres J, Di Marzo V (2007) Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int J Obes 31(4):692
32. Doris JM, Millar SA, Idris I, O'sullivan SE (2019) Genetic polymorphisms of the endocannabinoid system in obesity and diabetes. Diabetes Obes Metab 21(2):382–387
33. Zeng J, Li J, Huang G (2011) 385 C/A polymorphism of the fatty acid amide hydrolase gene is associated with metabolic syndrome in the Chinese Han population. Arch Med Sci: AMS 7(3):423
34. De Luis D, Sagrado MG, Aller R, Izaola O, Conde R, Romero E (2010) 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 88(1):76–80

35. Aberle J, Fedderwitz I, Klages N, George E, Beil F (2007) Genetic variation in two proteins of the endocannabinoid system and their influence on body mass index and metabolism under low fat diet. Horm Metab Res 39(05):395–397
36. Chiang KP, Gerber AL, Sipe JC, Cravatt BF (2004) Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet 13(18):2113–2119
37. de Moraes Martins CJ, Genelhu V, Pimentel MMG, Celoria BMJ, Mangia RF, Aveta T, Silvestri C, Di Marzo V, Francischetti EA (2015) Circulating endocannabinoids and the polymorphism 385C>A in fatty acid amide hydrolase (FAAH) gene may identify the obesity phenotype related to cardiometabolic risk: a study conducted in a Brazilian population of complex interethnic admixture. PLoS ONE 10(11):e0142728
38. Touriño C, Oveisi F, Lockney J, Piomelli D, Maldonado R (2010) FAAH deficiency promotes energy storage and enhances the motivation for food. Int J Obes 34(3):557
39. Li C, Jones PM, Persaud SJ (2011) Role of the endocannabinoid system in food intake, energy homeostasis and regulation of the endocrine pancreas. Pharmacol Ther 129(3):307–320
40. Egertova M, Cravatt B, Elphick M (2003) Comparative analysis of fatty acid amide hydrolase and CB1 cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. Neuroscience 119(2):481–496