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A genetic variant of the *NAMPT* gene rs4730153 as a risk factor for the metabolic syndrome in younger age: a single-centre pilot study in Yogyakarta, Indonesia

Anggelia Puspasari[1,2*], Pramudji Hastuti[1], Ahmad Hamim Sadewa[1], Rosdiana Mus[1,3], Citra Maharani[1,2] and Ika Setyawati[4]

Abstract

Background: The genetic variation of *nicotinamide phosphoribosyl transferase* (*NAMPT*) gene rs4730153 is reported to be associated with cardiometabolic risk, but the results are inconsistent between populations. Ethnicity, metabolic risk and lifestyle play a role in the association of the genetic variant and the metabolic syndrome (MetS). To the best of our knowledge, no research has yet been published concerning the Javanese population, so this study aimed to investigate the association of rs4730153 with MetS and its interaction with metabolic risk and lifestyle.

Results: The GG genotype ($p = 0.031$; OR 95% CI 3.88 [1.13–13.33]), GA+GG genotype ($p = 0.048$; OR 95% CI 10.52 [1.02–108.01]) and G allele carrier ($p = 0.006$; OR 95% CI 4.19 [1.51–11.64]) of rs4730153 had a higher risk of the MetS after adjusting for obesity, hypercholesterolemia, smoking and food intake. The risk was statistically significant for the younger age group ≤ 45 years old.

Conclusion: The GG, GA+GG genotype and G allele carrier of rs4730153 have a higher risk of the MetS, especially those who are obese, hypercholesterolemic and smokers and have a higher food intake in those aged ≤ 45 years old. Further larger, multicentre studies are required to confirm these pilot results.

Keywords: Genetic variant, Metabolic syndrome, rs4730153, *NAMPT*

Background

The prevalence of metabolic syndrome (MetS) continues to increase worldwide, including in Indonesia [1], and is associated with increased mortality and morbidity rates caused by cardiovascular diseases [2, 3]. MetS is a multifactorial disease influenced by interactions between genetic variations, lifestyle and other factors, with genetic variation estimated to contribute more than 20% to the MetS phenotype. Genetic variation mapping has recently gained attention for disease pathophysiology and interventions [2, 4].

Chronic inflammation processes are involved in MetS pathophysiology, including the increased release of pro-inflammatory cytokines especially from white adipose tissue (WAT) [2]. Visfatin encoded by the *NAMPT* gene is a pro-inflammatory cytokine mainly released from WAT and has an important role in lipid metabolism, carbohydrate metabolism, adipocyte plasticity and endothelial dysfunction and is immunomodulatory [5]. A meta-analysis reported that plasma Visfatin levels are associated with MetS, diabetes mellitus, atherosclerosis and other metabolic abnormalities [6].

A single nucleotide polymorphism (SNP) located in the intronic region gene can alter protein function and

* Correspondence: Anggelia.puspasari@unja.ac.id
[1]Department of Biochemistry, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Department of Medical Biology and Biochemistry, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia
Full list of author information is available at the end of the article

level, although the functional study of *NAMPT* genetic variant rs4730153 has not been fully elucidated. An epidemiology study in Saudi Arabia reported that the genotype variant *NAMPT* gene rs4730153 is associated with plasma Visfatin, with subjects with GG and GA genotypes having higher plasma Visfatin than the AA genotype [7].

A previous study reported that the genetic variant of rs4730153 is associated with cardiometabolic risk. A genotype-phenotype association study related to cardiometabolic risk in Spain showed that the AA genotype has a protective effect on cardiovascular risk [8]. Furthermore, in Chinese obese children, the GG genotype has a protective effect against abnormality markers of lipid and glucose metabolism [9]. The two studies reported different protective alleles and both of them focused on genotype and phenotype association but did not analyse any other modifying factors. Therefore, this pilot study investigated the association of rs4730153 with MetS and its interaction with other modifying factors (metabolic risk and lifestyle) in a Javanese living in Yogyakarta, Indonesia.

Methods

Study design and subject recruitment

A case-control study of 168 Javanese subjects aged 20–66 years living in the Yogyakarta region was conducted, with the case and control subjects carefully matched by gender and age. The population was screened to identify MetS patients in Yogyakarta, Indonesia, in 2018–2019. The inclusion criteria of this study were Javanese, aged 20–66 years old, living at Yogyakarta region at least for 5 years. Pregnant women and subjects with signs of active infection and history of cancer and taking antihypertension, lipid-lowering and antidiabetic agents regularly for at least 3 months were excluded from this study. Subjects with at least three criteria of a MetS diagnosis based on the *National Cholesterol Education Program-Adult Panel Treatment* (NCEP-ATP) III criteria modification for an Asian population were selected as the case group and the others were the control group.

All study participants provided written informed consent. The study protocol was according to the Declaration of Helsinki and was approved by our institution Medical and Health Research Ethics Committee (approval number KE/0621/05/2018).

Anthropometric, blood pressure, metabolic parameters and food intake measurement

Waist circumference was measured with a standard tape measure with an anatomical marker mid-circumference between the lowest rib and anterior superior iliac spine. Central obesity was defined as waist circumference ≥ 90 cm for men and ≥ 80 cm for women. Subjects with a body mass index (BMI) higher than 30 kg/m^2 were classified as obese. Systolic and diastolic blood pressures were measured twice in a seated position after 5 min of rest using a calibrated sphygmomanometer. Blood peripheral samples (5 mL) were obtained from the antecubital vein after 8 h of fasting. Subjects with a systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg were classified as hypertensive.

Plasma glucose, HDL, triglycerides and total cholesterol were measured by colorimetric enzymatic methods adapted for auto-analysis (Cobas $c111^R$ analyser according to the glucose HK, HDL cholesterol Gen4, triglycerides and cholesterol Gen2 protocols of Roche diagnostics ^{R}; Germany). Hyper-glycemia was defined as fasting plasma glucose ≥ 100 mg/dL, hyper-triglycerides were defined as fasting plasma triglycerides ≥ 150 mg/dL and hyper-cholesterol was defined as total cholesterol ≥ 200 mg/dL. The low-HDL group was defined as plasma HDL < 40 mg/dL for men or plasma HDL < 50 mg/dL for women; otherwise, it was high-HDL. Smokers were defined as smoking at least one cigarette/day for 1 year in their lifetime; otherwise, they were considered as non-smokers. Food intake data were collected based on subjects' food records for 3 days a week, 2 weekdays and 1 day at the weekend. All subjects were informed by a trained and experienced nutritionist how to record their food intake. Food record data were analysed with Nutri-survey software by a trained and experienced nutritionist.

Genotyping

Deoxyribonucleic acid (DNA) was extracted from the venous blood buffy-coat solid-phase using a proteinase K DNA extraction kit (FavorPrep^R). The purity and concentration were determined via the Nano-drop with a cut-off of ~ 1.8 for purity.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was performed for genotyping [7, 10], and products were visualised by electrophoresis at 100 V in a 3% agarose gel for 45 min. The primers, digestion enzymes and digestion products for both genetic variants are shown in Table 1. The PCR conditions were 94 °C for 7 min as initial denaturation and 1 min denaturation, followed by annealing at 60 °C

Table 1 PCR-RFLP primers, digestion enzymes and digestion products

Primer	Digestion enzyme	Digestion product
rs4730153		
F:5′-AATTGGGTAAGGTATGGTTGA-3′ R:5′CAGATTTACTTAGGCAGACACTTGA-3′	RsaI	G allele, 100 and 67 bps A allele, 167 bps

for 1 min and extension at 72 °C for 1 min, with a final extension of 7 min.

Data analysis

The distribution of the continuous data scale was assessed by Kolmogorov-Smirnov and non-normally distributed data were log 10 transformed. Data not normally distributed after transformation are presented as median (minimal-maximum) and the Mann-Whitney test was used. Normally distributed data are presented as mean ± standard deviation and the independent *t*-test was used for analysis. The association between the genetic variant and MetS and its diagnosed components was analysed by bivariate and multivariate analyses, with Pearson chi-square and Fisher tests used for bivariate analysis. Predictive logistic regression models were used for multivariate analysis adjusted for age, gender, obesity, hyper-cholesterol, smoking status and food intake data. The Hardy-Weinberg equilibrium (HWE) was tested.

Results

Baseline sample characteristics

The characteristics of the study participants are shown in Table 2. Gender proportion for case and control groups was similar, while the case subjects were older but this did not reach significance.

Waist circumference, BMI, blood pressure, plasma fasting glucose, triglycerides and total cholesterol were significantly higher in the MetS group than in the control group, whereas HDL plasma was significantly lower in the MetS group than in the controls. There were more smokers in the MetS group but this was not statistically insignificant.

The proportion of obese subjects was higher in the MetS group than in the control group (86.7% [*n* = 39] vs. 13.3% [*n* = 6]). Also, more subjects had hypercholesterol in the MetS group than in the control group (71.4% [*n* = 20] vs. 28.6% [*n* = 8]). Total calories, carbohydrates, fat and protein intake were higher in the MetS than in the control group but not statistically significant.

Genotyping distribution

The GG genotype of rs4730153 was the most frequent genotype, with the G allele being the wild type and the A allele was the minor allele. The minor allele frequency of rs4730153 was 28% in this study population and the goodness of fit test showed deviation from the HWE (Table 3).

Association of the genetic variant with MetS

The association of MetS with the genetic variants was statistically insignificant with and without age stratification according to the bivariate analysis (Table 4). Since age stratification was based on the median age of the study subjects and for biological reasons, 45 years old was used as the cut-off point.

Bivariate analysis showed subjects with the GA, GG genotype and G allele of rs4730153 had a greater risk of MetS than the AA genotype in the age group ≤ 45 years, while the recessive (GG/GA vs AA) and dominant (GG vs AA/GA) model showed a similar trend. Differences in the risk of genotypes were found in the age group > 45 years, whereby the GA, GG and G allele carrier had a

Table 2 Baseline subject characteristics

Characteristic	MetS (*n* = 84)	Control (*n* = 84)	*p*-value
Age (years)	46.15 (22.06–65.7)	45.66 (20.46–65.85)	1.000[a]
Sex (female), *n* (%)	47 (55.9)	47 (55.9)	1.000[c]
BMI (kg/m^2)	29.9 ± 4.71	23.94 ± 3.51	< 0.001[a]
Waist circumference (cm)	95.97 ± 10.39	81.04 ± 8.8	< 0.001[a]
Systolic blood pressure (mmHg)	130 (100–190)	110 (90–155)	< 0.001[b]
Diastolic blood pressure (mmHg)	85 (60–145)	75 (60–95)	< 0.001[b]
Plasma fasting glucose (mg/dL)	71.05 (44.05–227.6)	68.8 (43.2–110.4)	0.021[b]
Plasma fasting HDL (mg/dL)	36.70 ± 6.61	43.22 ± 10.05	< 0.001[a]
Plasma fasting triglyceride (mg/dL)	184.28 ± 93.41	101.57 ± 41.62	< 0.001[a]
Total cholesterol (mg/dL)	184.1 (88.1–380.2)	159.3 (95.3–250.2)	< 0.001[b]
Smokers, *n* (%)	21 (59.5)	15 (40.5)	0.259[c]
Total calories (kcal)	1203.3 (803.4–2582.4)	1178.5 (759.70–2014.4)	0.503[b]
Carbohydrates (g)	167.43 ± 49.84	164.44 ± 46.79	0.433[a]
Fat (g)	50.79 ± 20.69	46.70 ± 16.30	0.168[a]
Protein (g)	44.40 ± 14.94	42.72 ± 13.26	0.433[a]

[a]Independent *t*-test, [b]Mann-Whitney test, [c]Pearson chi-square, $p < 0.05$

Table 3 Genotype distribution of the genetic variance of *NAMPT* rs4730153

Genotype	Observed value	Expected value	X^2 (DF)	*p*-value	MAF
rs4730153					
AA	24	13			
AG	47	68	16.17	3.1×10^{-4}	0.28
GG	97	86			

X^2 value with degrees of freedom (DF) = 2. *MAF* minor allele frequency

lower odds ratio for MetS than the AA genotype. The association of genetic variance for rs4730153 and MetS was not statistically significant in both age groups (Table 4).

The multivariate analysis results are shown in Table 5. Logistic regression analysis of the codominant, recessive and dominant genotype model and allele analysis was statistically significant with an odds ratio between 3.88 and 16.14. This model inserted obesity, hypercholesterolemia, smoking status, protein intake and fat intake as modifiers, which were selected based on $p > 0.25$ in the bivariate analysis for age ≤ 45 years.

Discussion

This study investigated the role of genetic variants of Visfatin rs4730153 as risk factors for MetS. The GG genotype, GG+GA genotype and G allele carrier of rs4730153 were risk factors for MetS in interaction with modifying risk factors of obesity, hypercholesterolemia, smoking and food intake, especially in younger age in our population. Furthermore, our results confirm that MetS is multifactorial and that metabolic risk and food intake can modify genetic and MetS association.

A previous study reported that genetic variants of Visfatin rs4730153 played a role in the cardiometabolic risk in Segovia, Spain, with the GG genotype increases the risk for cardiovascular disease assessed with FRAMINGHAM and systematic coronary risk evaluation (SCORE) [8]. Both of these scores included blood pressure and HDL, metabolic risks that were also assessed in the NCEP-ATP III MetS diagnosis. Similarly, a study in China also reported obesity as a risk factor for MetS associated with the GG genotype [11]. Different genotype risks were reported in Han Chinese obese children, where the GG genotype was found to be a protective genotype against hyper-triglycerides [9]. Differences in the genotype risk between populations may be due to ethnicity, the criteria for subject recruitment or environmental factors.

The physiological mechanisms and how these genetic variants contribute to metabolic abnormalities remain unclear. However, an epidemiology study in Saudi Arabia found subjects with GG and GA genotypes had higher plasma Visfatin than the AA genotype [7], which supports the finding that GG genotype is a risk factor for diseases related to higher plasma Visfatin.

Higher plasma levels of Visfatin are associated with its role as a pro-inflammatory cytokine through the activated nuclear factor (NF) κB pathway. The upregulation of Visfatin promotes the release of other pro-inflammatory

Table 4 Association of the genetic variance of *NAMPT* rs4730153 with MetS stratified for age

rs4730153	Age ≤ 45 years old				Age > 45 years old			
	MetS ***n* = 40**	***Control*** ***n* = 40**	***p*-value**	**OR (95% CI)**	***MetS*** ***n* = 44**	***Control*** ***n* = 44**	***p*-value**	**OR (95% CI)**
Genotype								
Additive model								
AA	3	7	ref		9	5	ref	
GA	9	11	0.350[b]	1.91 (0.38–9.6)	13	14	0.326[a]	0.516 (0.14–1.9)
GG	28	22	**0.124[b]**	2.97 (0.69–12.8)	22	25	0.251[a]	0.49 (0.14–1.68)
Recessive/dominant model								
AA	3	7	ref		9	5	ref	
GG+GA	37	33	**0.176[a]**	2.61 (0.63–10.95)	35	39	0.244[a]	0.500 (0.15–1.63)
AA+GA	12	18	ref		22	29	ref	
GG	28	22	**0.166[a]**	1.91 (0.76–4.79)	22	25	0.521[a]	0.76 (0.33–1.76)
A allele	15	25	**ref**	1.97	31	24	ref	0.69
G allele	65	55	**0.068[a]**	(0.94–4.1)	57	64	0.255[a]	(0.36–1.31)

[a]Chi-square test; [b]Fischer exact test. *MetS* metabolic syndrome, *OR* odds ratio with 95% CI, *ref* reference genotype

Table 5 Multivariate analysis of the association of the genetic variance of *NAMPT* rs4730153 with MetS and modifying factors for subjects aged ≤ 45 years

rs4730153 for age ≤ 45	Logistic regression model			
	B	**SE**	***p*-value**	**Adjusted OR (95% CI)**
Genotype				
Additive model				
AA			ref	
GA			NS	
GG	2.78	1.34	**0.039**	16.14 (1.16–225.21)
Recessive/dominant model				
AA			ref	
GG+GA	2.35	1.19	**0.048**	10.52 (1.02–108.01)
AA+GA			ref	
GG	1.36	0.63	**0.031**	3.88 (1.13–13.33)
Allele				
A allele			**ref**	4.19
G allele	1.43	0.52	**0.006**	(1.51–11.64)

Hosmer and Lemeshow test was performed to analyse the goodness of fit; *p*-value for all models was > 0.05. Logistic regression model inserting obesity, hypercholesterolemia, smoking and food intake as modifiers. *B* logistic regression model coefficient, *NS* non-significant, *SE* standard error, *OR* odds ratio with 95% CI, *ref* reference genotype

cytokines and has a role in the positive feedback of chronic inflammation, a component of MetS pathophysiology that can alter lipid metabolism, carbohydrate metabolism and adipocyte plasticity. Furthermore, it can lead to cardiometabolic abnormalities [12–16]. The NF-κB pathway induced by the increase in Visfatin increases the production of inducible nitrite oxide synthase (iNOS) which leads to endothelial dysfunction, which is also associated with cardiovascular risk, MetS and hypertension [17, 18].

Age stratification was performed in this study based on the Indonesian Health Ministry for determining age groups of adults, pre-elderly and elderly. Participants aged 22–45 years old (younger) were in the adult group, while subjects aged 46–66 years old (older) were in the pre-elderly and elderly groups. Differences in the metabolic profile related to ageing are influenced by hormones, body fat composition, metabolic rate and other environmental factors. These differences may also influence the genetic phenotype association [19, 20]. Also, Visfatin plasma levels decline with age [21], which may temper the impact of *NAMPT* genetic variants on metabolic risk of phenotype in older age. The association of the *NAMPT* genetic variant was more prominent with metabolic risk in the younger group which was also found in a Chinese population as well as in our study [9, 11].

The minor allele frequency (MAF) of rs4730153 (0.28) in this study was higher than other studies conducted in Asia (0.08–0.11) but lower than studies in Europe (0.42) or South America (0.50) [8, 10, 11, 22]. The G allele was reported as wild type, which was the same for all studies. The deviation from HWE in our population may be related to the small sample size. Other studies also reported a deviation of HWE in the *NAMPT* genetic variant in their populations [10, 23]. We performed PCR-RFLP for genotyping, an established, reliable and affordable method for genotyping, especially when resources are limited [24, 25].

This case-control pilot study of a Javanese, especially those who lived in the Yogyakarta region, although small, the number of participants met minimal quota sampling which was calculated based on the hypothesis for an odds ratio for a case-control study. Two large epidemiology studies reported that the prevalence of MetS in Indonesia varies from 21.66 to 39.0% [26, 27]. The study which reported a comparison of the prevalence of MetS among ethnic groups and regions in Indonesia reported a prevalence of MetS in Yogyakarta of 15.4% and the Javanese of 19.85%, lower than the national prevalence [27]. This difference may influence the age of the study participants, time of surveillance performed and methods for diagnosis of MetS component. The laboratory measurement of the two studies used capillary blood but did not mention if the blood sample was fasting and one study did not measure triglycerides [26, 27]. Capillary blood for the measurement of blood glucose and cholesterol is more affordable for large surveillance but not the gold standard for blood glucose, cholesterol and triglycerides. Our study used plasma from the peripheral blood vein collected after 8–10 h of fasting, which is the gold standard for measurement of the metabolite profile.

To the best of our knowledge, this is the first study of the Javanese population who live in Yogyakarta, Indonesia, that reported genetic variants of the *NAMPT* gene are associated with MetS. However, MetS is a multifactorial disease, with polygenic interactions other than the *NAMPT* gene, epigenetic regulation, other lifestyle and environmental factors contribute to the pathophysiology. The case and control groups were carefully age- and sex-matched and dietary intake was recorded to reduce research bias. Some limitations of our study were the small sample size and the lack of measurement of plasma Visfatin and physical activities.

Conclusion

The GG, GA+GG genotype and G allele carrier of *NAMPT* rs4730153 have a higher risk for suffering MetS in our study population, interacting with other metabolic risks, smoking and food intake, especially in younger age groups. Further larger, multicentre and multifactorial (genetic and non-genetic) studies are required to confirm these results.

Abbreviations

BMI: Body mass index; DNA: Deoxyribonucleic acid; HDL: High-density lipoprotein; HWE: Hardy-Weinberg equilibrium; iNOS: Nitrite oxide synthase; MAF: Minor allele frequency; MetS: Metabolic syndrome; *NAMPT*: Nicotinamide phosphoribosyltransferase; NCEP-ATP: *National Cholesterol Education Program-Adult Panel Treatment*; NF: Nuclear factor; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; SNP: Single nucleotide polymorphism; SCORE: Systematic coronary risk evaluation; WAT: White adipose tissue

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Authors' contributions

All authors contributed to this research. AP, RM and CM were involved in collecting the participant data, sample and laboratory analysis. AP, IS, PH and AHM performed and interpreted the data and manuscript editing. All authors read and approved the final manuscript.

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Availability of data and materials

The data sets analysed during the current study are not publicly available due to subject confidential agreement but are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was arranged based on the Helsinki Declaration and had approval from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Dr. Sardjito Public Hospital, Yogyakarta, Indonesia. Ethical approval number was KE/0621/05/2018. All subjects who participated signed informed consent forms after receiving detailed information concerning the purpose of the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Biochemistry, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. [2]Department of Medical Biology and Biochemistry, Faculty of Medicine and Health Sciences, Universitas Jambi, Jambi, Indonesia. [3]Diploma Degree Technology of Medical Laboratory, Faculty of Health Technology, Universitas Mega Rezky, Makassar, Indonesia. [4]Department of Biochemistry, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Yogyakarta, Indonesia.

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