Original Article
The 1012C>G polymorphism of nucleobindin 2 is associated with the development of coronary artery disease

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Abstract: Objective: Nucleobindin 2 (NUCB2) is a precursor of nesfatin-1 which is a hypothalamic anorectic neuropeptide. Nesfatin-1 is found to be involved in the pathogenesis of coronary artery disease (CAD). Our study is designed to examine whether the 1012C>G polymorphism of NUCB2 gene is associated with an increased risk of developing CAD in Chinese Han population. Methods: This study consisted of 285 patients with CAD and 117 healthy subjects. Polymerase chain reaction and direct sequencing method was utilized to examine 1012C>G polymorphism of NUCB2 gene in this population. Results: CG and GG genotype frequencies of NUCB2 polymorphism was significantly reduced in CAD patients than those in healthy controls. Furthermore, there is lower G allele frequency in CAD patients compared with healthy controls. Conclusions: 1012C>G polymorphism of NUCB2 gene is associated with the development of CAD in Chinese Han population.

Keywords: Polymorphism, nucleobindin 2, nesfatin-1, coronary artery disease

Introduction

Coronary artery disease (CAD) is a major cause of mortality worldwide. Insulin resistance, dyslipidemia, hypertension, family history, obesity, and smoking are considered to be traditional risk factors for CAD development [1]. In addition, genetic factors also contribute to the incidence of CAD [2]. A variety of studies have focused on the genetic susceptibility to CAD and found that more and more gene polymorphisms are associated with an increased risk of developing CAD [3].

Nucleobindin 2 (NUCB2), is a precursor of nesfatin-1 which is a neuropeptide widely expressed in the central nervous system. Nesfatin-1 regulates appetite and energy metabolism. Intracerebroventricular injection of nesfatin-1 caused decreased nocturnal food intake and body weight gain [4]. Nesfatin-1 plays an important role in the mechanism of cardiovascular diseases including CAD. Nesfatin-1 depressed the contractility and relaxation of cardiomyocytes and limited ischemia/reperfusion damage, acting in post-conditioning protection [5]. Dai et al. reported that plasma nesfatin-1 levels were significantly lower in acute myocardial infarction group control group [6]. Recently, a genetic polymorphism (1012C>G) in NUCB2 gene was found in obese populations [7, 8]. It is plausible that this genetic variant in NUCB2 gene may influence an individual's susceptibility to CAD.

The aim of this study is to examine the association of the 1012C>G polymorphism of NUCB2 gene with the risk of CAD in Chinese Han population.

Materials and methods

Subjects

A consecutive population of 285 patients with angiographically proven CAD was enrolled in our study. CAD was defined as subjects who had angiographic evidence of stenosis ≥50% in

Table 1. Anthropometric and metabolic characteristics parameters of the patients with CAD and control subjects

Characteristics	CAD (n=285)	Control (n=117)	*P*
Age (years)	60.65±9.84	59.21±10.62	0.195
Gender (M/F)	136/106	49/37	0.901
BMI (kg/m^2)	24.69±2.97	24.41±2.35	0.370
SBP (mmHg)	142.66±14.42	124.02±13.98	<0.001
DBP (mmHg)	84.23±10.29	82.06±8.96	0.047
TG (mmol/L)	1.67±0.91	1.08±0.47	<0.001
TC (mmol/L)	5.12±1.09	4.97±0.93	0.203
HDL-C (mmol/L)	1.10±0.22	1.52±0.22	<0.001
LDL-C (mmol/L)	3.45±0.73	2.99±0.44	<0.001

Data are given as mean ± SD. Abbreviations: CAD, coronary artery disease; 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.

at least one major coronary artery. The control group consisted of 117 age- and sex-matched subjects who were recruited from individuals attending the routine check up in our hospital. All control subjects were free of personal or family history of cardiovascular or hemorrhagic disease. The study protocol was approved by the Human Ethics Review Committee of our hospital and a signed consent form was obtained from each subject.

Measurements

Weight, height, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. Venous blood was obtained in the fasting state at 7:00 am after overnight. Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by auto biochemistry instrument (Hitachi 7170, Tokyo, Japan). Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters (kg m^{-2}).

DNA genotyping

Blood samples were collected from 285 patients with CAD and 117 healthy subjects. Genomic DNA from all the subjects was extracted from peripheral blood leukocytes using a DNA isolation kit following the manufacturer's instructions (Qiagen). Genotyping of G1359A polymorphism of CNR1 was performed using polymerase chain reaction and then direct sequencing.

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 software. Data are presented as means ± standard deviation (SD). Biochemical variables were compared between case and control group using the Student t-test. A chi square test was performed to examine Hardy-Weinberg equilibrium. The genotype and allele frequencies were compared using the χ^2 test. Two-tailed P<0.05 was considered statistically significant.

Results

The baseline parameters

As shown in **Table 1**, CAD group showed higher levels of SBP, DBP, TG, LDL-C, and lowered HDL-C level than the control subjects. No significant differences were observed in other characteristics between the two groups.

Association of the polymorphism with the development of CAD

The genotype frequencies of 1012C>G polymorphism among the controls were in agreement with Hardy-Weinberg equilibrium (*P*= 0.853). As presented in **Table 2**, CG and GG genotype frequencies of NUCB2 polymorphism was significantly reduced in CAD patients than those in healthy controls. Furthermore, there is lower G allele frequency in CAD patients compared with healthy controls.

Discussion

Nesfatin-1 is an 82-amino acid protein encoded by the nucleobindin 2 gene. Nesfatin-1 is expressed in several hypothalamic nuclei and extra-hypothalamic areas [9]. Nesfatin-1 plays an important role in the cardiovascular function by regulating blood pressure. Intracerebroventricular administration of nesfatin-1 could interact with the central melanocortin system to increase sympathetic nerve activity and lead to an increase in mean arterial pressure in conscious rats [9]. Mimee et al. reported that microinjection of nesfatin-1 into the medial

Table 2. Distribution of CNR1 gene polymorphism among the subjects with CAD and control subjects

Genotype	CAD group (n=285)		Control group (n=117)		P value
	N	%	N	%	
CC	131	46.0	42	35.9	0.008
CG	136	47.7	57	48.7	
GG	18	6.3	18	15.4	
C	398	69.8	141	60.3	0.009
G	172	30.2	93	39.7	

nucleus of the solitary tract induced significant increases in blood pressure [10]. Chronic peripheral nesfatin-1 administration also increased blood pressure in rats [11]. Recently, 1012C>G polymorphism of NUCB2 gene is demonstrated to be correlated with blood pressure [12].

Nesfatin-1 also regulates cardiac function. Nesfatin-1 protein and NUCB2 mRNA were expressed in rat cardiac extracts. Exogenous nesfatin-1 depressed heart contractility and relaxation without affecting coronary motility [13]. Microinjection of nesfatin-1 into nucleus ambiguus activated cardiac vagal neurons of nucleus ambiguus and elicited bradycardia in conscious rats [14]. Nesfatin-1 protected against cardiac ischemia/reperfusion injury by reducing infarct size, lactate dehydrogenase release, and post-ischemic contracture [13]. Plasma nesfatin-1 levels were significantly lower in acute myocardial infarction group control group [6]. These results indicate the important role of nesfatin-1 in cardiac function and possible protective effects in the development of CAD. Our investigation found a significant association of NUCB2 polymorphism with the occurrence of CAD. Therefore, NUCB2 polymorphism may serve as a genetic risk factor for CAD. Subjects with CC genotype are considered to be risk population for CAD and should perform some preventive strategies such as changing life style, weight loss, or statin treatment.

Obesity is a clear risk factor for CAD. Obesity also increases the risk for developing traditional CAD risk factors including hypertension, dyslipidemia, and diabetes mellitus, resulting in a greater occurrence of CAD. Numerous investigations have demonstrated the association of obesity with angina, myocardial infarction (MI),

heart failure, and sudden cardiac death [15]. Nesfatin-1 is a peptide closely correlated with appetite and obesity [16]. Intracerebroventricular injection of nesfatin-1 caused decreased nocturnal food intake and body weight gain [4]. Recent studies have found reduced plasma nesfatin-1 concentrations in obese patients [4]. In addition, an association of 1012C>G polymorphism of NUCB2 gene with obesity was observed recently [7, 8]. Therefore, this polymorphism may contribute to the development of CAD through obesity-related mechanism.

It should be mentioned, however, that the current study inevitably has some limitations. First, this study had a relatively small population size. Second, as our study was only conducted in a sample of Chinese Han population, the result is lack of replication in other population. Therefore, our data should be validated in prospective studies with a larger population size in other ethnic populations.

Our data provides evidence for the first time that the 1012C>G polymorphism of NUCB2 gene is associated with an increased risk of CAD in the Chinese Han population. Further studies are necessary to determine the exact mechanism by which this polymorphism could influence the risk of developing CAD.

Disclosure of conflict of interest

None.

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References

[1] Mack M, Gopal A. Epidemiology, traditional and novel risk factors in coronary artery disease. Cardiol Clin 2014; 32: 323-332.

[2] Roberts R. Genetics of coronary artery disease. Circ Res 2014; 114: 1890-1903.

[3] Dandona S, Roberts R. The role of genetic risk factors in coronary artery disease. Curr Cardiol Rep 2014; 16: 479.

[4] Oh-I S, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 2006; 443: 709-712.

[5] Imbrogno S, Angelone T, Cerra MC. Nesfatin-1 and the cardiovascular system: central and pheripheral actions and cardioprotection. Curr Drug Targets 2015; 16: 877-883.

[6] Dai H, Li X, He T, Wang Y, Wang Z, Wang S, Xing M, Sun W, Ding H. Decreased plasma nesfatin-1 levels in patients with acute myocardial infarction. Peptides 2013; 46: 167-171.

[7] Chen YY, Chan RM, Tan KM, Poh LK, Loke KY, Wang JP, Li H, Hu YH, Wang L, Lee KO, Li GW, Lee YS. The association of a nucleobindin 2 gene (NUCB2) variant with childhood adiposity. Gene 2013; 516: 48-52.

[8] Zegers D, Beckers S, Mertens IL, Van Gaal LF, Van Hul W. Association between polymorphisms of the Nesfatin gene, NUCB2, and obesity in men. Mol Genet Metab 2011; 103: 282-286.

[9] Yosten GL, Samson WK. Nesfatin-1 exerts cardiovascular actions in brain: possible interaction with the central melanocortin system. Am J Physiol Regul Integr Comp Physiol 2009; 297: R330-336.

[10] Mimee A, Smith PM, Ferguson AV. Nesfatin-1 influences the excitability of neurons in the nucleus of the solitary tract and regulates cardiovascular function. Am J Physiol Regul Integr Comp Physiol 2012; 302: R1297-1304.

[11] Ayada C, Turgut G, Turgut S, Güçlü Z. The effect of chronic peripheral nesfatin-1 application on blood pressure in normal and chronic restraint stressed rats: related with circulating level of blood pressure regulators. Gen Physiol Biophys 2015; 34: 81-88.

[12] Tragante V, Barnes MR, Ganesh SK, Lanktree MB, Guo W, Franceschini N, Smith EN, Johnson T, Holmes MV, Padmanabhan S, Karczewski KJ, Almoguera B, Barnard J, Baumert J, Chang YP, Elbers CC, Farrall M, Fischer ME, Gaunt TR, Gho JM, Gieger C, Goel A, Gong Y, Isaacs A, Kleber ME, Mateo Leach I, McDonough CW, Meijs MF, Melander O, Nelson CP, Nolte IM, Pankratz N, Price TS, Shaffer J, Shah S, Tomaszewski M, van der Most PJ, Van Iperen EP, Vonk JM, Witkowska K, Wong CO, Zhang L, Beitelshees AL, Berenson GS, Bhatt DL, Brown M, Burt A, Cooper-DeHoff RM, Connell JM, Cruickshanks KJ, Curtis SP, Davey-Smith G, Delles C, Gansevoort RT, Guo X, Haiqing S, Hastie CE, Hofker MH, Hovingh GK, Kim DS, Kirkland SA, Klein BE, Klein R, Li YR, Maiwald S, Newton-Cheh C, O'Brien ET, Onland-Moret NC, Palmas W, Parsa A, Penninx BW, Pettinger M, Vasan RS, Ranchalis JE, M Ridker P, Rose LM, Sever P, Shimbo D, Steele L, Stolk RP, Thorand B, Trip MD, van Duijn CM, Verschuren WM, Wijmenga C, Wyatt S, Young JH, Zwinderman AH, Bezzina CR, Boerwinkle E, Casas JP, Caulfield MJ, Chakravarti A, Chasman DI, Davidson KW, Doevendans PA, Dominiczak AF, FitzGerald GA, Gums JG, Fornage M, Hakonarson H, Halder I, Hillege HL, Illig T, Jarvik GP, Johnson JA, Kastelein JJ, Koenig W, Kumari M, März W, Murray SS, O'Connell JR, Oldehinkel AJ, Pankow JS, Rader DJ, Redline S, Reilly MP, Schadt EE, Kottke-Marchant K, Snieder H, Snyder M, Stanton AV, Tobin MD, Uitterlinden AG, van der Harst P, van der Schouw YT, Samani NJ, Watkins H, Johnson AD, Reiner AP, Zhu X, de Bakker PI, Levy D, Asselbergs FW, Munroe PB, Keating BJ. Gene-centric meta-analysis in 87,736 individuals of European ancestry identifies multiple blood-pressure-related loci. Am J Hum Genet 2014; 94: 349-360.

[13] Angelone T, Filice E, Pasqua T, Amodio N, Galluccio M, Montesanti G, Quintieri AM, Cerra MC. Nesfatin-1 as a novel cardiac peptide: identification, functional characterization, and protection against ischemia/reperfusion injury. Cell Mol Life Sci 2013; 70: 495-509.

[14] Brailoiu GC, Deliu E, Tica AA, Rabinowitz JE, Tilley DG, Benamar K, Koch WJ, Brailoiu E. Nesfatin-1 activates cardiac vagal neurons of nucleus ambiguus and elicits bradycardia in conscious rats. J Neurochem 2013; 126: 739-748.

[15] Jahangir E, De Schutter A, Lavie CJ. The relationship between obesity and coronary artery disease. Transl Res 2014; 164: 336-344.

[16] Abaci A, Catli G, Anik A, Kume T, Bober E. The relation of serum nesfatin-1 level with metabolic and clinical parameters in obese and healthy children. Pediatr Diabetes 2013; 14: 189-195.