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RESEARCH PAPER

Association of neuropeptide Y gene rs16147 polymorphism with metabolic syndrome in patients with documented coronary artery disease

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Abstract

Background and aims: There have been few epidemiological studies that have investigated genetic susceptibility to cardiovascular risk associated with the prevalence of metabolic syndrome (MetS). Neuropeptide Y (NPY) is a strong candidate gene for coronary artery disease (CAD). Therefore, the aim of this study was to investigate the association between the NPY gene rs16147 polymorphism and the presence of MetS in a well defined group of Iranian subjects with angiographically-defined CAD.

Methods: A cross-sectional study design was used in which a total of 364 patients were recruited; 143 patients with MetS and 221 without MetS were genotyped using the ARMS-PCR technique. Logistic regression analyses were performed to determine the odds ratios (ORs) for the association of specific genotypes with the presence of MetS and related phenotypes.

Results: The frequency of the variant G allele of the NPY gene was significantly higher in CAD patients without MetS ($p = 0.032$). Compared to the AA genotype of the NPY gene, individuals carrying the GG genotype had a reduced risk of MetS (OR = 0.51, 95% CI = 0.27–0.95, $p = 0.034$).

Conclusion: The rs16147 polymorphism may be associated with presence of MetS among subjects with documented CAD. Carriage of NPY A allele in patients with CAD is associated with a higher prevalence of MetS.

Keywords

Coronary artery disease, metabolic syndrome, neuropeptide Y gene polymorphism

History

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Introduction

Metabolic syndrome (MetS) is characterized by a cluster of clinical and biochemical features that include: central adiposity, hypertension, insulin resistance, hypertriglyceridemia, dyslipidemia and impaired glucose tolerance. There has been some debate about the existence of metabolic syndrome as a useful clinical entity and this is further complicated by the many criteria that have been used to define it (Sattar et al., 2008). It is likely that the aetiology of this syndrome is multifactorial, due to an interaction between several genetic and environmental factors (Bruce & Hanson, 2010). Identifying the risk factors associated with this syndrome is critical for planning future health policy and prevention strategies. MetS is thought to contribute to risk of atherosclerosis, type II diabetes mellitus (T2DM) and coronary artery disease (CAD) (Eckel et al., 2005). CAD is the main cause of death and a major cause of morbidity and loss of quality-of-life. Metabolic syndrome may be a link between diabetes and cardiovascular disease (Shahbazian et al., 2013). Prevalence of MetS is increasing in many regions including in Asia and several developing countries (Lameira et al., 2008; Pan et al., 2008). Its prevalence has been reported to be between 12.8–41.1% in different parts of the world (Ramachandran et al., 2003) and during the past few decades there has been a dramatic increase within the Iranian population, from ~10–63.2% (Kelishadi et al., 2008; Nezhad et al., 2008; Sarrafzadegan et al., 2008).

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter; it is expressed by noradrenergic neurons (both central and autonomic) as well as chromaffin cells (Menyhert et al., 2006; Sitticharoon et al., 2013; Turi et al., 2003;

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Zhang et al., 2012). Increased NPY signalling due to elevated NPY expression in the hypothalamus has been proposed to contribute to the development of obesity, type II diabetes and cardiovascular disease (Aberle et al., 2008; Campbell et al., 2007; Erkkila et al., 2002; Patel et al., 2006; Ukkola & Kesaniemi, 2007). Evidence for the impact of NPY gene variation on body weight regulation and risk of obesity has recently been reported (Ding et al., 2005; Mitchell et al., 2008).

The NPY gene, containing four exons, is located at 7p15.1 and, for the reasons discussed above, may be a promising candidate gene contributing to MetS susceptibility. The rs16174 (G-399A) polymorphism is located within the promoter region upstream of the NPY gene (Domschke et al., 2008; Heilig et al., 2004; Lindberg et al., 2006). This polymorphism is associated with a change in NPY expression and appears to be responsible for more than half of the variation in the expression of NPY *in vivo* (Buckland et al., 2005; Drevets et al., 2008). It has been shown to have a significant relationship with circulating concentrations of neuropeptide Y (Sommer et al., 2010; Zhou et al., 2008).

The association between the NPY rs16147 SNP polymorphism and the presence of MetS in individuals with co-existent CAD has not been studied extensively and, to our knowledge, there is little information about the genetic susceptibility to metabolic syndrome in the Iranian population. Given that MetS increases the risk of cardiovascular disease, this prospective study was designed to investigate the relationship between the rs16147 SNP of the NPY gene and the presence of metabolic syndrome in a group of Iranian subjects with angiographically-defined CAD.

Materials and methods

Study population

A cross-sectional design was used to assess the relationship between rs16147 NPY variant and the presence of MetS in patients with CAD. The study group consisted of 364 subjects with angiographically defined CAD, aged between 18–75 years. These patients were selected from individuals who underwent coronary angiography, in most cases for the investigation of stable angina, at Ghaem Medical Center, Mashhad, Iran. Patients who had systematic or infectious diseases or who were on treatment with oral contraceptives or hormone replacement therapy as well as pregnant women and those with a history of past angiography were excluded from the study. After analysis of the coronary angiograms by a Specialist Cardiologist, the presence of one or more stenoses >50% in diameter of at least one major coronary artery was considered as evidence of significant CAD. Subjects who had <50% reduction of coronary artery diameter were excluded from the study. MetS was defined by the International Diabetes Federation criteria (Alberti et al., 2005): systolic blood pressure of $\geq$130 mm Hg, diastolic blood pressure of $\geq$85 mm Hg, triglycerides of at least 150 mg/dL ($\geq$1.70 mmol/L), HDL-C less than 40 mg/dL (<1.03 mmol/L) for men and less than 50 mg/dL (<1.29 mmol/L) for women, fasting plasma glucose of at least 100 mg/dL ($\geq$5.60 mmol/L) and waist circumference greater than 94 cm for men and greater than 80 cm for women. According to IDF criteria, among 364 subjects, 143 had MetS and 221 did not. Subsequently, all these samples were included in the analyses of the rs16147 polymorphism of the NPY gene. All patients gave written consent and the study was approved by the Ethics Committee of Mashhad University of Medical Sciences.

Anthropometric and other measurements

Anthropometric parameters of individuals including weight, height, BMI, waist circumference, hip circumference and waist/hip ratio as well as systolic and diastolic blood pressures were measured as previously described (Ghayour-Mobarhan et al., 2008). A full fasting serum lipid profile including total cholesterol (TC), HDL, LDL and TG and fasting blood glucose (FBS) concentrations were measured by standard enzymatic techniques.

DNA extraction and genotyping

Peripheral blood was obtained from the subjects and the genomic DNA was extracted using commercial kit according to the manufacturer's protocol (Biogene Company, Tehran, Iran). Genotyping for the rs16147 polymorphism was performed by polymerase chain reaction-amplification refractory mutation system (ARMS-PCR) analysis. The forward and two reverse primer pair set used for amplification of the promoter region of NPY gene were 5′-CGT CTG AGC GAG TAC TTG AGG-3′ and 5′-CCT GCC AAC AGG ACT ACG AA-3′ and 5′-CCT GCC AAC AGG ACT ACG AG-3′. Because of the large difference between the forward and reverse primers annealing temperature in PCR, we performed ARMS-PCR twice for each sample. Each sample was tested using a forward and reverse primer which has a G nucleotide and reverse primer which has an A nucleotide separately. The ARMS-PCR reaction was performed in 20 µl final volume, using 2 µl Buffer, 0.4 µl dNTP mix, 1.5 µl $MgCl_2$, 1 µl for each primer, 0.2 µl Taq Polymerase, 2 µl DNA, 0.5 µl for each primer of Beta-actin (SBS Genetech Co. Ltd, Beijing, China) (was used as an internal control to ensure that false negative results were not obtained) and 11.4 µl distilled water. The amplification conditions for the ARMS-PCR were 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 63 °C for 30 s, DNA extension at 72 °C for 30 s and the final extension at 72 °C for 10 min. All amplification cycles were performed in a PCR system Veriti 96 well thermocycler (Applied Biosystems, Foster City, CA). The PCR products were identified by 2% agarose gel electrophoresis using green viewer staining and visualized directly under ultraviolet illumination. Product sizes were determined with reference to a 100 base pair (bp) DNA ladder (Figure 1). Finally, a direct sequencing approach (Sequetech, Mountain View, CA) was used to confirm the genotypes obtained by ARMS-PCR for a sample of the subjects with different genotypes from each group.

Statistical analysis

All the data were analysed using SPSS for Windows™, version11.5 software package (SPSS Inc., Chicago, IL). Data were expressed as means ± SD for normally distributed data or median and interquartile range for non-normally distributed data. The statistical difference in genotype distribution

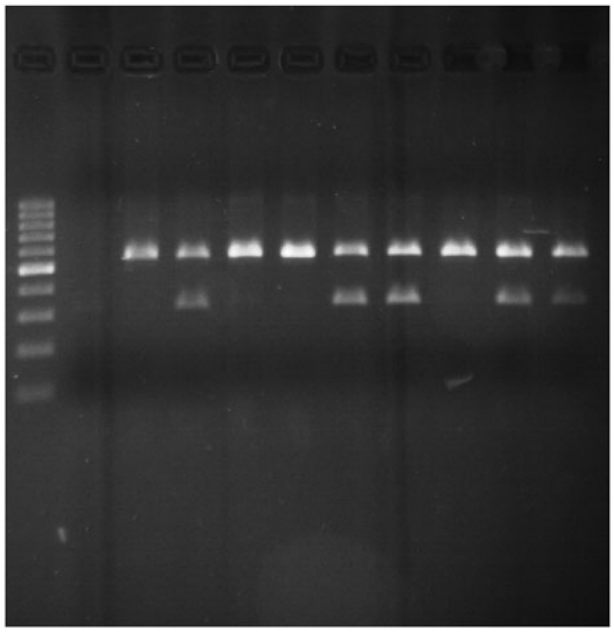

Figure 1. ARMS-PCR of the rs16147 polymorphism of different individuals based on polyacrylamide gel electrophoresis 2% stained with ethidium bromide. MK: 100 bp DNA ladder. Lane 1: sample without DNA. Lane 2: sample plus Beta-actin. Lanes 3 and 4: we used a primer which revealed the A allele. So, this pattern belongs to an individual who has AA homozygote genotype. Lanes 5 and 6: We applied a primer which showed the G allele. So, they are homozygote for GG genotype, the same as lane 7 and 8. Lanes 9 and 10: we used both primers here. This pattern related to heterozygote for AG genotype. Both A and G allele have the same molecular weight.

Table 1. Demographic, anthropometric and biochemical characteristics of the study groups.

Variables	MetS ($n=143$)	Non-MetS ($n=221$)	p Value
Age (years)	58.02 ± 10.1	58.05 ± 11.3	0.441
Gender			0.001
Men (%)	67 (46.9)	167 (74.9)	0.033
Women (%)	76 (53.1)	56 (25.1)	0.050
Height (cm)	159.01 ± 10.07	160.01 ± 10.1	0.005
Weight (kg)	72.23 ± 14.12	68.93 ± 12.22	0.001
BMI (kg/m^2)	30.02 ± 9.47	26.02 ± 6.17	0.001
Waist circumference (cm)	97.44 ± 10.18	87.07 ± 11.19	0.001
Hip circumference (cm)	100.62 ± 10.04	92.51 ± 12.24	0.001
TC (mg/dl)	180 ± 49	172 ± 42	0.032
TG (mg/dl)	172 ± 94	131 ± 75	0.001
HDL_C (MG/dl)	41 ± 10	44 ± 12	0.001
LDL_C (mg/dl)	101 ± 26	99 ± 32	0.357
FBS (mg/dl)	134 ± 59	108 ± 46	0.001
SBP (mm Hg)	111.3 ± 50.2	90.2 ± 49	0.201
DBP (mm Hg)	77.9 ± 25.3	63.2 ± 31.1	0.001
Hypertension	59.3%	42.7%	0.001
Obesity	91.3%	47.1%	0.001
History of diabetes mellitus	39.3%	18.2%	0.001

MetS, metabolic syndrome; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBS, fasting blood sugar; SBP, systolic blood pressure; DBP, diastolic blood pressure; SD, standard deviation; ANOVA, analysis of variance.

Values are expressed as mean ± SD or median and interquartile range. Comparisons were made by the χ^2 test (for categorical data), one-way ANOVA and Kruskal–Wallis test (for numerical data).

and allele frequencies between groups and analysis of deviation from the Hardy–Weinberg equilibrium were assessed using the chi-square or fisher exact test. Other variables were compared using one-way ANOVA (for normally-distributed variables) or Kruskal–Wallis test (for non-normally distributed variables). Post-hoc tests were used to analyse the significance between groups. Logistic-regression analyses were used to calculate odds ratio (OR) and 95% confidence interval (CI) to estimate the association of the rs16147 polymorphism with the risk of MetS. A two-sided p value <0.05 was considered statistically significant.

Results

Baseline characteristics

The baseline characteristics of study subjects are presented in Table 1. According to the results of demographic and metabolic characteristics, the percentage of individuals who had MetS was 23.1% and 46.2% in male and female sub-groups, respectively. The frequency of MetS observed in females was significantly higher compared with the male individuals ($p<0.001$). As would be expected, apart from high-density lipoprotein cholesterol (HDL-C), which was significantly lower ($p<0.001$), there was a significantly higher mean weight, BMI, waist circumference, hip circumference, total cholesterol (TC), triglyceride (TG), FBS and diastolic blood pressure (DBP) in the sub-group with MetS compared with the group without (all $p<0.05$). There was no significant difference in the low-density lipoprotein cholesterol (LDL-C) and systolic blood pressure between the patients with and without MetS ($p>0.05$).

Association between NPY rs16147 polymorphism and MetS

Results for the genotype distribution and allelic frequencies of the NPY rs16147 polymorphism in study groups are shown in Table 2. Three hundred and sixty-four individuals were genotyped for the NPY rs16147 polymorphism. The frequencies of AA, AG and GG genotypes were 21.7%, 53.8% and 24.5% in the MetS and 15.4%, 50.2% and 34.4% in the non-MetS group, respectively. The genotype distributions were in accordance with Hardy–Weinberg equilibrium in the MetS and in the non-MetS groups ($p>0.05$ for each group). Although the results of chi-square test showed no significant difference in genotype distribution between the two groups ($p=0.084$), logistic regression analysis indicated that, compared with the AA genotype of NPY gene, subjects carrying the GG variant genotype were associated with a decreased risk of MetS (OR = 0.51, 95% CI = 0.27–0.95, $p=0.034$). Similar values were obtained after adjusting for age, sex, family history of diabetes mellitus and smoking. The allele frequencies of the rs16147A>G polymorphism in CAD patients with and without metabolic syndrome were 48.6%/51.4% and 40.5%/59.5%, respectively. In logistic regression analysis, the variant G allele of the NPY rs16147 polymorphism showed significantly lower risk (which was parallelled by an increased risk of the A allele) for MetS (OR = 0.72, 95% CI = 0.53–0.97, $p=0.032$).

Table 2. Genotype distribution of the NPY rs16147 polymorphism in individuals with coronary artery disease with and without co-existing metabolic syndrome.

	CAD patients					
Genotype/allele	With MetS ($n=143$)	Without MetS ($n=221$)	Crude OR (95% CI) MetS vs Non-metS	p Value	OR (95% CI)† MetS vs Non-metS	p Value†
AA	31 (21.7%)	34 (15.4%)	–	–	–	–
AG	77 (53.8%)	111 (50.2%)	0.74 (0.43–1.34)	0.345	0.84 (0.45–1.56)	0.573
GG	35 (24.5%)	76 (34.4%)	0.51 (0.27–0.95)	0.034	0.56 (0.28–1.13)	0.105
	$p=0.084$					
A allele	139 (48.6)	179 (40.5)	–			
G allele	147 (51.4)	263 (59.5)	0.72 (0.53–0.97)	0.032		

χ^2-test and logistic regression were used. Populations were in accordance to the Hardy–Weinberg equilibrium. OR, odds ratio; CI, confidence interval. †Adjusted for age, sex, family history of diabetes mellitus and smoking.

Table 3. Genotype distribution of NPY rs16147 polymorphism in subjects with different components of metabolic syndrome.

		Central obesity		Hyperglycemia		Dyslipidemia		Hypertension		HDL	
NPY	Genotype	Yes	No	Yes	No	Yes	No	Yes	No	Low	Normal
rs16147	AA	42 (21.2)	22 (14.0)	21 (20.6)	48 (17.7)	30 (19.4)	33 (16.3)	37 (27.3)	31 (16.1)	16 (22.2)	38 (15.6)
	GA	107 (54.0)	75 (47.8)	53 (52.0)	138 (50.9)	85 (54.8)	99 (48.8)	97 (54.2)	93 (48.4)	33 (45.8)	130 (53.3)
	GG	49 (24.7)	60 (38.2)	28 (27.5)	85 (31.4)	40 (25.8)	71 (35.0)	45 (25.1)	68 (35.4)	23 (31.9)	76 (31.1)
	p Value	0.015		0.697		0.174		0.089		0.359	

Values are expressed as number (%) for genotypes. Comparisons were made by the χ^2 test.

Association of neuropeptide Y rs16147 ploymorphism with baseline features of MetS

The genotype distribution of NPY rs16147 polymorphism with individual components of MetS including central obesity, elevated triglycerides, hyperglycaemia, low HDL-C and hypertension is presented in Table 3. Subjects carrying the rs16147 GG variant genotype were found to be at decreased risk of central obesity ($p=0.015$). There were no significant differences between the genotype groups with respect to other features of MetS. In fact, no significant difference in NPY rs16147 polymorphism has been showed in hypertension vs normal blood pressure, dyslipidemic vs non-dyslipidemic, diabetes mellitus vs non-diabetic and low-HDL vs normal-HDL groups ($p>0.05$).

The group distribution of rs16147 genotypes were also analysed according to anthropometric and biochemical parameters and the results showed no statistically significant differences in their mean scores in CAD patients with and without metabolic syndrome (data not shown).

Discussion

The neuropeptide Y gene is highly polymorphic and several polymorphisms of the gene have been found to be associated with adiposity or obesity phenotypes, such as high body mass index (Patel et al., 2006; Sitticharoon et al., 2013; Zhang et al., 2012). The overall goal of the present cross-sectional study was to investigate the association of the NPY rs16147 polymorphism with MetS and its features as risk factors for CAD. Although the association of NPY rs16147 polymorphism with stable angiographic coronary artery disease is clear (Lindberg et al., 2006; Shah et al., 2009; Shine et al., 1994; Sommer et al., 2010), there has been no previous study investigating its relationship with MetS. Therefore we conducted this cross-sectional study to evaluate the relationship between an uncommon polymorphism in the NPY gene with the prevalence of MetS in Iranian patients with CAD.

The present study is the first one in an Iranian population which shows that the NPY rs16147 polymorphism was associated with an altered risk of metabolic syndrome among CAD patients. Our study demonstrated that there was a significant association between the NPY rs16147 genotypes and the presence of CAD with and without co-existing metabolic syndrome in an Iranian population. The GG homozygotes had significantly decreased the risk of MetS when compared with the A allele carriers (AA and AG) in the CAD patients (OR = 0.51, 95% CI = 0.27–0.95, $p=0.034$). In other words, a 49% decreased risk of MetS was observed in subjects with the rs16147 GG genotypes compared with the AA carriers, suggesting that the G allele might confer a protective effect against MetS.

We also evaluated the association of the rs16147 polymorphism with individual components of MetS including central obesity, elevated triglycerides, hyperglycaemia, low HDL-C and hypertension. Our results showed that the rs16147 GG genotype was found to be strongly associated with decreased central obesity, suggesting the allele-specific effects of the rs16147 polymorphism on NPY gene expression and possible involvement of the A allele on susceptibility to obesity and metabolic syndrome. With respect to anthropometric indexes, lipid profiles and glucose levels in CAD patients with and without MetS, no differences were seen between the genotype distributions in both groups.

Promoter polymorphisms, especially those involved in transcription regulation such as transcription factor (TF) binding can affect gene expression (Kim et al., 2009). The SNP rs16147 is a functional polymorphism located in the

NPY gene promoter region which has shown an allele-specific effect on NPY expression and accounts for the majority of the variation in plasma NPY peptide levels (Buckland et al., 2004; Itokawa et al., 2003; Shah et al., 2009; Zhou et al., 2008). It has been reported that this SNP is due to the loss of Sp1 (a transcription factor) binding consensus by substitution of G to A and that it may affect NPY expression and NPY peptide level (Itokawa et al., 2003; Shah et al., 2009), but recent *in silico* analysis by Kim et al. (2009) revealed no allele-specific effect on expression level. Therefore, according to Kim et al. (2009), the effect of the rs16147 polymorphism on NPY expression may come from the interaction of G/A allele with other regulatory genomic DNA regions or involvement of other TFs than Sp1.

NPY has been considered as a thrifty gene due to its role in weight regulation and energy balance (Rohner-Jeanrenaud & Jeanrenaud, 1997). The ''thrifty gene'' hypothesis implicates an evolutionary selection for metabolic genes for the development of Metabolic Syndrome in the condition of overnutrition and sedentary lifestyle. With this in mind, some reports speculate that the NPY rs16147 polymorphism might affect the expression of the NPY gene, resulting in changes in NPY peptide level, which can stimulate sympathetic nervous system activity, contributing to an increase in blood pressure level as a component of metabolic syndrome, as well as inducing vasoconstriction and stimulating vascular smooth muscle cell proliferation and angiogenesis, supporting the possible role in development of CAD, obesity and metabolic syndrome (Baltatzi et al., 2008; Pons et al., 2004; Zhang et al., 2012).

To our knowledge, this is the first report to suggest an association of this uncommon polymorphism with MetS. The strength of our study is that cases were carefully selected from a well-defined and ethnically homogenous population sample which avoided effects of any apparent acute or chronic inflammatory disease or infections. However, there are several potential limitations. First, we only analysed one SNP, the rs16147 polymorphism, so it is not adequate to conclude that genetic variability in NPY is associated with MetS or its related phenotypes. Another limitation is the fact that we have looked specifically among patients with CAD and, hence, it may not be possible for these results to be generalized to the whole population and the data should be extrapolated to other regions and ethnic groups cautiously. Finally, the functional relevance of the polymorphism with plasma NPY concentrations according to rs16147 genotypes remains to be determined in future studies in our population.

Conclusions

In conclusion, our study suggests for the first time that the NPY rs16147 promoter polymorphism may be associated with the presence of MetS among subjects with documented CAD in an Iranian northeast population. Carriage of NPY A allele in patients with CAD is associated with high prevalence of MetS. However, further studies should be replicated with separate study subjects and/or other ethnic subjects to investigate a more subtle effect of this gene in this serious phenotype and to explore the underlying mechanisms of our findings.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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