ORIGINAL ARTICLE

Influence of Neuropeptide Y and Neuropeptide Y 2 Receptor Variants in Acute Coronary Syndrome

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Abstract

Background: The neuropeptide Y (NPY) is one of the most abundant neurotransmitters in the nervous system. NPY acts as a potent stimulator of angiogenesis, inflammation, and adipogenesis, through the NPY 2 receptor (NPY2R). Changes in the NPY signaling pathway have been linked to Acute Coronary Syndrome (ACS).

Objectives: The purpose of this study is to determine the association between variants in the NPY and NPY2R genes, as well as the severity of acute coronary syndrome (ACS).

Methods: Approximately 221 ACS patients and 278 healthy controls were selected for this study. Four variants in NPY and two variants in NPY2R genes were genotyped using Taqman allelic discrimination and sequencing. The Chi-square and Fisher's exact tests were used to verify the genotype frequencies. The logistic regression analyses were used for the evaluation of the studied variables. Haplotype analysis was used to evaluate the linkage disequilibrium (LD) between the variants ($p<0.05$).

Results: An association of NPY c.20T>C variant was found with the ACS group when compared to the healthy group. In the analysis between variants and risk factors in the ACS group, NPY c.84G>A was associated with hypertension. The analysis between TIMI risk showed a significance for NPY c.20T>C between the low and intermediate/high TIMI risk groups. In the haplotype analysis, strong linkage disequilibrium (LD) was found between the variants NPY c.150G>A and NPY c.-485T>C.

Conclusion: The NPY c.20T>C variant appears to contribute to the development of ACS. The NPY2R c.-1116A>G variant may contribute to the early development of ACS and the NPY c.84G>A variant appears to contribute to the development of hypertension. In addition, the NPY c.20T>C is associated with a protective effect in ACS severity.

Keywords: Acute Coronary Syndrome; Neuropeptide Y; Receptors Neuropeptide Y; Nucleotide Polymorphism; Epidemiology.

Introduction

The neuropeptide Y (NPY) is a small peptide with 36 amino acids and is one of the most abundant neurotransmitters of the central and peripheral nervous system.[1] It induces proliferation of vascular smooth muscle cells in humans, promoting the formation and development of new blood vessels.[2] As a result, current research is focused on developing a drug delivery mechanism for NPY to prolong the therapy of diabetic cardiomyopathy and ischemic heart disease without significant systemic consequences.[3] In mammals, NPY

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acts through its receptors NPYR1, NPYR2, NPY4R, NPY5R, and NPY6R. The NPY1R, NPY2R, NPY4R, and NPY5R receptors are functional in all mammals, but Y6 is a pseudo-gene in humans and other primates, and is also absent in the mouse genome.[4] The NPY c.20T>C mutation, which results in the substitution of Leucine for Proline (Leu7Pro) in the pre-pro-NPY signal peptide, is associated with an increase in serum lipid levels. NPY c.20T>C increased the risk of accelerated and early progression of atherosclerosis,[5-7] acute myocardial infarction and stroke,[8] coronary artery disease,[9] hypertension,[10,11] and obesity in children.[12] The NPY c.84G>A and c.150G>A variants were investigated for their association with obesity and hypertension,[10-11] while c.150G>A was studied for its association with atherosclerosis.[7]

By contrast, the NPY c.-485T>C (rs16147) variant, which is located in the promoter region,[13] has proven to alter NPY expression in vitro, and is likely to affect mRNA expression levels in vivo.[14-15] Numerous studies have established a link between the c.-485T>C variant and the development of early atherosclerosis[7] and stroke.[16-17] Other studies have established a link between this variant and a reduction of insulin resistance and type 2 diabetes.[18-19] Through the NPY 2 receptor (NPY2R), NPY may also act as a stimulator of angiogenesis, inflammation, and adipogenesis in the abdominal region.[20] NPY2R variants c.-1088C>T and c.-1116A>G are associated with obesity,[21-24] as well as with low HDL-C serum levels.[25] Since the earliest experimental studies with NPY, its association with cardiovascular diseases has been established, with the evidence that cardiac NPY is released from sympathetic nerves during acute myocardial infarction.[26] Plasma levels of NPY were found to be high in human suffering from Acute Coronary Syndromes (ACS), such as acute myocardial infarction and during left ventricular failure.[27-29] NPY and the NPY2R may serve as biomarkers for ACS prognosis, risk stratification for death or cardiovascular events, or even a potential therapeutic target in other types of treatments. This purpose of this study was to evaluate the influence of NPY and NPY2R variants on the severity of ACS.

Methods

Population

The study enrolled patients who were admitted to the Real Heart Hospital (RHH), which is affiliated with the Royal Portuguese Charity Hospital in Pernambuco (RPHCP), located in Recife, Pernambuco, Brazil. Adult ACS patients (n = 221), over 18 years of age, of both sexes, underwent an electrocardiogram, and if necessary, cineangiocardiography was also performed. The myocardial injury markers, creatine kinase-MB (CK-MB) and troponin, were measured. Non-ACS patients admitted to the RHH (n = 95) with atrial fibrillation were also included in this study. The sample size was defined by convenience. A total of 278 healthy blood donors (healthy group), over 18 years of age, of both sexes, were recruited from the Hematology and Hemotherapy Foundation of Pernambuco (Hemope) and participated in interviews on the presence of acute or chronic diseases or significant comorbidities and laboratory tests (HIV, hepatitis C, syphilis, Human T-lymphotropic virus type 1 and 2, and Chagas' disease) to identify infectious parasitic diseases.

Inclusion criteria

This study enrolled patients who were diagnosed with ACS, after having undergone a physical examination, an electrocardiogram, and a measurement of myocardial injury markers. The non-ACS patients were those hospitalized without a diagnosis of coronary disease and typically who presented atrial fibrillation.

Exclusion Criteria

Individuals taking anti-inflammatory drugs, those who had suffered a recent trauma, those with a history of active infectious diseases or neoplasms, and those who refused to participate in the study were excluded from the ACS and non-ACS groups.

Ethical considerations

The Real Heart Hospital/Realcor Research Ethics Committee of the Royal Portuguese Charity Hospital in Pernambuco approved this study (CAAE: 03187512.2.0000.5202). Before sample collection, each subject was informed about the research and signed an informed consent form.

Level of ACS severity in patients

In all patients, the severity level was determined by the left ventricular ejection fraction, which was determined by the echocardiogram. Additionally, the risk score for Thrombolysis in Myocardial Infarction (TIMI) was used. The TIMI Risk Score is used to categorize patients as having an intermediate and high risk of suffering

a cardiovascular event. For the patients without the ST-elevation myocardial infarction, seven risk factors were considered.[30] For the patients with an ST-elevation myocardial infarction (STEMI), eight factors are considered, in accordance with Morrow et al. (2000).[31]

Blood samples and DNA extraction

During the routine of cardiologic exams performed on RPHCP, peripheral blood samples were collected in a 5ml EDTA tube to perform molecular analysis at the Oswaldo Cruz Foundation (Fiocruz-PE). Purification of the DNA was carried out according to manufacturer's instructions, using the Illustra blood genomicPrep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK).

Identification and genotyping of variants

DNA sequencing was used to screen for the NPY gene variants 20T>C (rs16139), c.84G>A (rs5572), and c.150G>A (rs5573). Initial denaturation at 95°C for 5 minutes was followed by 35 cycles of denaturation (95°C for 1 minute), annealing (60°C for 30 seconds), and extension (72°C for 45 seconds), followed by a final extension at 72°C for 7 minutes32. According to manufacturer's recommendations, Platinum® Taq DNA polymerase (Invitrogen Life Technologies) was used. The reagents were used without adding DNA as a negative control. The amplified fragments were viewed on a 1.5% agarose gel. The PCR products were sequenced and analyzed using the Chromas Lite 2.01 program at the Aggeu Magalhes Institute's Technological Platform Centers (NPT/IAM). The NPY variants c.-485T>C (rs16147) (Assay ID C 2267279 10), NPY2R c.-1088C>T (rs6857715) (Assay ID C 29013142 10), and NPY2R c.-1116A>G (rs6857530) (Assay ID C 44837 30) were identified by applying real-time PCR with Genotyping, which was carried out using the 7500 RealTime PCR System (Applied Biosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation (92°C for 15 seconds), annealing (60°C for 60 seconds), extension (60°C for 60 seconds), and final extension at 60°C for 60 seconds.

Statistical analysis

To determine whether the genotypic frequencies agree with the Hardy-Weinberg proportions, the Chi-square and Fisher's exact tests were used. The clinical variables, inflammatory markers, ischemia, and variants were all evaluated using logistic regression analyses. The data were analyzed using The R Project for Statistical Computing (R Development Core Team), version 2.10. The haplotype analysis and evaluation of linkage disequilibrium (LD) between variants were performed using the HaploView 4.2 software. When the p-value was less than 0.05, the data were considered statistically significant.

Results

Characterization of the study population

A total of 221 patients with ACS were selected, the majority of whom (76.02%) was male. This distribution was also observed for non-ACS patient and health patient groups, comprised of 52.69% (49/93) female individuals and 84.17% (234/278) male individuals, respectively. The median age ACS, non-ACS, and healthy patients was respectively 60, 6,3 and 45 years. No significant differences in age were found between the ACS and non-ACS groups ($p = 0.7775$), but significant differences were observed between the ACS and the healthy groups ($p < 0.001$). According to the TIMI risk, patients with ACS were classified as having a low risk (34.84%), an intermediate risk (46.61%), or a high risk (18.55%). Multiple logistic regression analysis revealed that gender, smoking, diabetes, and dyslipidemia are contributing factors to the increased risk of ACS (Supplementary Table S1).

Analysis of the variants

The groups of patients with ACS and healthy patients showed Hardy-Weinberg equilibrium (HWE) ($p > 0.05$) for all variants in NPY and NPY2R. In the group of non-ACS patients, NPY c.150G>A, NPY2R c.-1088C>T, and c.-1116A>G showed HWE, while NPY c.20T>C and NPY c.84G>A were not in HWE. Therefore, no statistical analyses involved these two populations.

The NPY c.20T>C showed a majority of ancestral homozygous genotype TT (94.57%) in the ACS group, while 5.43% of the patients were heterozygous. In the healthy group, 96.40% of the samples showed the TT genotype, while only 3.60% presented the TC or CC genotypes. When the analysis after adjusting for age and gender was performed, there was an association between this variant and ACS. Previously, it presented $p = 0.3251$ for the TC/CC and $p = 0.3307$ for the C allele, later showing a $p = 0.0256$ for the TC/CC and $p = 0.0892$ for the C allele, with an odds ratio (OR) of approximately three-fold that of the individual having the TC/CC genotype or the C allele to develop ACS, as can be seen in Table 1.

Table 1 – Analysis of the variants of the genes in the ACS and healthy groups

Variants	Healthy		ACS		OR	95% CI		p	$OR_{adjusted}$	95% CI		p
	N*	%	N (221)	%		Inf	Sup			Inf	Sup	
NPY c.20T>C (rs16139)												
Genotype												
TT	268	96.4	209	94.57	1.00				1.00			
TC/CC	10	3.6	12	5.43	1.54	0.65	3.71	0.3251	3.29	1,15	9.52	**0.0256**
Alleles												
T	546	98.2	430	97.29					1.00			
C	10	1.8	12	2.71	1.52	0.65	3.64	0.3307	2.17	0.89	5.40	0.0892
NPY c.84G>A (rs5572)												
Genotype												
GG	264	94.96	207	93.67	1.00				1.00			
GA/AA	14	5.04	14	6.33	1.28	0.59	2.76	0.5320	1.66	0.61	4.46	0.3185
Alleles												
G	542	97.48	427	96.6	1.00				1.00			
A	14	2.52	15	3.4	1.36	0.64	2.87	0.415	1.47	0.66	3.35	0.3506
NPY c.150G>A (rs5573)												
Genotype												
GG	76	27.34	55	24.89	1.00				1.00			
GA/AA	202	72.66	166	75.11	1.14	0.76	1.70	0.5366	1.30	0.75	2.28	0.3568
Alleles												
G	289	51.98	220	49.77	1.00				1.00			
A	267	48.02	222	50.23	1.09	0.85	1.40	0.4889	1.17	0.89	1.54	0.2592

NPY c.-485T>C (rs16147)**												
Genotype												
TT	31	24.22	58	26.61	1.00				1.00			
TC/CC	97	75.78	160	73.39	0.88	0.53	1.45	0.6240	0.75	0.40	1.41	0.3768
Alleles												
T	121	47.27	224	51.38	1.00				1.00			
C	135	52.73	212	48.62	0.85	0.62	1.16	0.2966	0.80	0.58	1.11	0.1790
NPY2R c.-1088T>C (rs6857715)												
Genotype												
TT	44	21.57	39	17.65	1.00				1.00			
TC/CC	160	78.43	182	82.35	1.28	0.79	2.08	0.3089	0.91	0.49	1.73	0.7823
Alleles												
T	201	49.26	188	42.53	1.00				1.00			
C	207	50.74	254	57.47	1.31	1.00	1.72	0.0493	1.31	0.99	1.75	0.0625
NPY2R c.-1116A> G (rs6857530)												
Genotype												
AA	46	21.5	74	33.48	1.00				1.00			
AG/GG	168	78.5	147	66.52	0.54	0.35	0.83	**0.0054**	0.81	0.46	1.44	0.4758
Alleles												
A	212	49.53	257	58.14	1.00				1.00			
G	216	50.47	185	41.86	0.71	0.54	0.92	**0.0109**	0.73	0.55	1.97	0.0286

Note: **The sample N of the variants of the healthy group varies (for NPY c.20T>C, NPY c.84G>A and NPY c.150G>A = 278; NPY c.-485T>C = 128; NPY2R c.-1088C> T = 204; NPY2R c.-1116A G = 214); **The sample N in the ACS group is 218; OR: Odds Ratio; OR-adjusted: Odds Ratio adjusted by age and gender; CI: Confidence Interval; p: p-value, obtained through the logistic regression test.*

Regarding NPY c.84G>A, the results show that 93.67% of ACS patients presented the GG genotype, while 6.33% showed GG or GA genotypes. Results showed that 94.96% of the samples from healthy individuals presented the GG genotype, while only 5.04% showed GA or AA genotypes. No statistical difference was found between the groups and no association between NPY c.84G>A and ACS was identified (Table 1).

Results from the NPY c.150G>A showed 24.89% of the patients with ACS carrying the GG genotype, while 75.11% of the patients showed GA/AA genotypes. Among the non-ACS patients, 32.97% showed the GG genotype, while 67.03% presented the GA/AA genotypes (Supplementary Table S2). The analysis of healthy individuals revealed that the GG genotype was present in 27.34% of the patients, while 49.28% presented the GA genotype and 23.38% the AA genotype (Table 1). No statistical difference was found between the groups and no association between NPY c.150G>A and ACS was identified.

As regards the NPY c.-485T>C variant, 26.61% of the ACS group presented the TT genotype, while 73.39% of these presented the TC/CC genotypes. Among the non-ACS patients, 25.81% presented the TT genotype, while 74.19% presented the TC/CC. No association was found between this variant and the ACS (Supplementary Table S2). In the healthy group, individuals presented 24.22% of the TT genotype, while 75.78% presented the TC/CC genotypes (Table 1). In the two analyses performed with the three populations, no association was found between c.-485T> C and ACS.

Regarding NPY2R c.-1088C>T, the genotypic distribution in the group of patients with ACS showed that 17.65% presented the TT genotype, while 82.35% presented the TC/CC genotypes. In the non-ACS patient group, 24.21% of the individuals showed the TT genotype, while 75.79% presented TC/CC genotypes (Supplementary Table S2). In the healthy group, only 21.57% of the individuals showed the TT genotype, while 78.43% presented the TC/CC genotypes (Table 1). In the two analyses performed with the three populations, no association was found between c.-485T>C and ACS.

The results regarding NPY2R c.-1116A>G showed a genotypic distribution in the ACS group of 33.48% presenting the CC genotype as compared to 66.52% presenting the AG/GG genotypes. In the non-ACS group, 26.32% of the patients showed the AA genotype, while 73.68% presented the AG/GG genotypes (Supplementary Table S2). In the healthy group, 21.50% showed the AA genotype, while 78.50% presented the AG/GG genotypes. When the logistic regression analysis was performed, an association between c.-1116A>G and ACS (p = 0.0054 for the AG/GG genotypes and p = 0.0109 for the G allele) was observed between the healthy and the ACS groups. After adjustment for age and gender, this association continued (p = 0.4619 for the AG/GG genotypes and p = 0.2982 for the G allele), as can be observed in Table 1.

Variants versus TIMI risk

TIMI risk showed an association with the NPY c.20T>C variant. The NPY c.20T>C presented a significant difference (p = 0.0261; OR = 0.25) among the low and intermediate/high TIMI risk groups. In the low TIMI risk group, the genotype distribution was 89.61% TT and 10.39% TC/CC, while in the intermediate/high TIMI risk group, 97.22% showed the TT genotype and 2.78% showed the TC/CC genotypes (Table 2). For the other variants, no association was found with any of the TIMI risk groups.

Relationship between variants and clinical and biological characteristics and habit

The association of the variants with the following variables of ACS was also analyzed: gender/time, smoking, diabetes, hypertension, dyslipidemia, levels of C-reactive protein (CRP), number of arterial lesions, troponin levels, levels of CK-MB mass, and ejection fraction.

The results showed that in the ACS group, the NPY c.84G>A variant was associated with hypertension, indicating a 3.57-fold chance of developing this disease (p = 0.0223) in individuals who do not have GA/AA genotypes (Table 3).

The NPY2R c.-1116A>G variant showed an association with the onset time of ACS (Table 4), indicating that the individual who has this variant has almost twice the chance to developing the early syndrome (p = 0.0253; OR = 1.91). However, no association was found for other variants (Supplementary Tables S3, S4, S5, and S6).

Haplotype analysis

The pairwise LD values (D' and r2) among the studied variants in the NPY gene are provided in the supplements. Strong LD was found between variants NPY c.150G>A and NPY c.-485T>C, which are separated by 41 kb (D'= 0.967; r2 = 0.909). However, a weak LD

Table 2 – Analysis of the variants of the genes in the different degrees of TIMI Risk

Variants (genotype)	TIMI Risk Score						
	Low (n = 77)		Intermediate/High * (n = 144)				
	N	%	n	%	OR	95% CI	p
NPY c.20T> C (rs16139)							
TT	69	89.61	140	97.22	1		
TC/CC	8	10.39	4	2.78	0.25	0.06 – 0.81	**0.0261**
NPY c.84G>A (rs5572)							
GG	71	92.21	136	94.44	1		
AG/AA	6	7.79	8	5.56	0.70	0.23 – 2.19	0.5173
NPY c.150G>A (rs5573)							
GG	22	28.57	33	22.92	1		
GA/AA	55	71.43	111	77.08	1.35	0.71 – 2.51	0.3551
NPY c.-485T>C (rs16147)**							
TT	21	27.63	37	26.06	1		
TC/CC	55	72.37	105	73.94	1.08	0.57 – 2.02	0.8020
NPY2R c.-1088T>C (rs6857715)							
TT	14	18.18	25	17.36	1		
TC/CC	63	81.82	119	82.64	1.06	0.50 – 2.15	0.5300
NPY2R c.-1116A> G (rs6857530)							
AA	20	25.97	54	37.50	1		
AG/GG	57	74.03	90	62.50	0.58	0.31 – 1.07	0.0852

Note: **Reference; **Sample of 77 from the low TIMI group and 144 on the Intermediate/High; OR: Odds Ratio; CI: Confidence Interval; p: p-value, obtained through the logistic regression test.*

was found between the NPY2R c.-1088C>T and NPY2R c.-1116A>G variants (Supplementary Figure S7).

Discussion

Genotyping of NPY and NPY2R polymorphisms in 221 ACS patients and 278 healthy controls indicated that the NPY c.20T>C polymorphism significantly raised the risk of ACS. The NPY2R c.-1116A>G contributes to the development of the early stages of the syndrome. In addition to this, the NPY c.20T>C is associated with a protective effect in ACS severity.

NPY has been linked to hypertension, congestive heart failure, and other cardiovascular diseases due to its high sympathetic nervous system activity.[33] The NPY c.20T>C variant has been linked to cardiovascular pathologies, such as accelerated and early progression of atherosclerosis,[5,6] acute myocardial infarction, and cerebrovascular disease in hypertensive patients.[8] The results of the present study were similar to those reported by Masoudi-Kazemabad et al.,[9] which showed an association between NPY c.20T>C and coronary artery disease in an Iranian population.[9] Wallerstedt et al.,[8] demonstrated the association of this variant with acute myocardial infarction in a Swedish hypertensive population.[8] The NPY c.20T>C variant was initially associated with increased cholesterol and low-density lipoprotein (LDL) levels,[5] in obese and healthy individuals.[6] In a subsequent study in Finnish women with coronary heart disease, the NPY c.20T>C is associated with total cholesterol, but not with LDL.[34] Further, the

Table 3 – Analysis of the variant NPY c.84G>A with habits and biological and clinical features

Variables	NPY c.84G>A (rs5572)				OR	95% CI		p
	GG		GA/AA					
	N	%	N	%		Inf	Sup	
Onset of ACS								
Prospective	95	45.89	4	28.57				
Premature	112	54.11	10	71.43	2.12	0.69	7.93	0.2162
Gender								
Male	156	75.36	12	85.71	1.00			
Female	51	24.64	2	14.29	0.51	0.08	1.95	0.3881
Premature								
Male	102	82.26	9	90				
Female	22	17.74	1	10	0.5152	0.03	2.95	0.539
Prospective								
Male	54	44.26	3	60	1.00			
Female	68	55.74	2	40	0.5294	0.07	3.30	0.4945
Gender/Time								
Male								
Prospective	54	34.62	3	25	1.00			
Premature	102	65.38	9	75	1.5882	0.45	7.38	0.501
Female								
Prospective	41	80.39	1	50	1.00			
Premature	10	19.61	1	50	4.1	0.15	109.92	0.333
Smoking								
No	150	72.46	12	85.71	1.00			
Yes	57	27.54	2	14.29	2.27	0.60	14.29	0.2903
Diabetes								
No	117	56.52	9	64.29	1.00			
Yes	90	43.48	5	35.71	1.38	0.46	4.55	0.5715
Hypertension								
No	45	21.74	7	50.00	1.00			
Yes	162	78.26	7	50.00	3.57	1.18	11.11	**0.0223**
Dyslipidemia								
No	84	40.58	5	35.71	1.00			
Yes	123	59.42	9	64.29	0.81	0.2427	2.4390	0.7198
Change in CRP								
No	65	34.76	6	46.15	1.00			
Yes	122	65.24	7	53.85	0.62	0.20	2.00	0.4100

ALN *								
0	11	5.79	2	14.29	1.00			
≤2	96	50.53	8	57.14	0.46	0.09	2.43	0.3597
>2	83	43.68	4	28.57	0.26	0.04	1.62	0.1503
TROPONIN I > 0.11 ng/ml								
No	30	14.63	1	7.14	1.00			
Yes	175	85.37	13	92.86	2.23	0.42	41.22	0.4481
CK-MB mass >5.6 ng/ml								
No	58	30.85	5	38.46	1.00			
Yes	130	69.15	8	61.54	0.71	0.23	2.45	0.5688
EF								
<50%	66	36.67	5	38.46	1.00			
≥50%	114	63.33	8	61.54	0.93	0.30	3.17	0.8969

Note: *C-reactive protein (CRP); Arteries Lesion Number (ALN); Ejection Fraction (EF); OR: Odds Ratio; CI: Confidence Interval; p: p-value, obtained through the logistic regression test. *Multinomial logistic regression.*

NPY c.20T>C is associated with altered lipid metabolism[18] and increased the susceptibility to type 2 diabetes mellitus (T2DM) and diabetic retinopathy in T2DM.[35] Bhaskar et al.,[11] evaluated the NPY c.20T>C variant with hypertension and found an association.[11] The variations in the associations are due to variations in allele frequencies across populations. The carrier frequency of the C allele of the NPY c.20T>C ranges from 6-15% in Caucasian populations, but it is very low or absent in Eastern populations.[36,3] The highest allele frequencies were found in the Nordic countries. Moreover, the NPY c.20T>C could originate in northern Europe and then spread to neighboring regions.[36] Our results showed no association between NPY variant c.84G>A and ACS, corroborating with Shah et al.,[7] who showed no existence of an association of this variant with the early onset of atherosclerosis in American and European populations. Regarding NPY c.150G>A, it was not associated with hypertension, corroborating Bhaskar et al.,[11] Studies with Korean and Chinese populations have shown that the variant c.-485T>C of NPY can be considered a genetic risk factor or be involved with stroke.[16,17] Likewise, Shah et al.,[7] demonstrated the association of this variant with the risk of developing early atherosclerosis. Our findings indicate that the NPY2R variant c.-1116A>G is associated with ACS. Although no studies corroborate our findings, the NPY2R variant c.-1116A>G has been associated with obesity in Caucasian Danes[23] and with HDL-C in Japanese populations.[25]

In the present study, pairwise LD values between NPY c.20T>C and c.150G>A variants indicated that there is no strong LD between these markers. These findings were corroborated by a few other studies that did not report a significant LD between these markers.[10,11] Further, a weak LD between the NPY c.20T>C and c.-485T>C variants was demonstrated in this study, corroborating with Patel et al.,[18] The possible explanation for the weak LD between these markers is due to the lack of recombination between adjacent markers or low frequency of the alleles.[3]

The study's primary limitation is that the evaluated population was from Brazil, a country well-known for its high genetic heterogeneity. Genetic diversity studies in five Brazilian geopolitical regions revealed that European ancestry contributed the most (77.1%), followed by African (14.3%) and Amerindian (8.5%) contributions.[38] Pena et al.,[39] also showed that the European ancestry was predominant in all regions studied in Brazil, with proportions ranging from 60.6% in the Northeast to 77.7% in the South. Further, Ferreira et al.,[40] demonstrated that 79% of contributions to a population in the state of São Paulo came from Europeans, 14% from Africans, and 7% from indigenous Brazilian Amerindians. Furthermore, the Rio de Janeiro population had a predominantly European genetic influence (from 55.2 to 58.6%), followed by African (from 31.1 to 30.3%) and Amerindian (from 13.7 to 11.0%) contributions.[41] Further, the number of individuals studied may limit the conclusions of our results.

Table 4 – Analysis of the variant NPY2R c.-1116A>G with habits and biological and clinical features

Variables	NPY2R c.-1116A> G (rs6857530)				OR	95% CI		p
	AA		AG/GG					
	N	%	N	%		Inf	Sup	
Onset of ACS								
Prospective	41	55.41	58	39.46	1.00			
Premature	33	44.59	89	60.54	1.91	1.09	3.37	**0.0253**
Gender								
Male	53	71.62	115	78.23	1.00			
Female	21	28.38	32	21.77	0.70	0.37	1.34	0.2787
Premature								
Male	30	78.95	81	82.65	1.00			
Female	8	21.05	17	17.35	0.787	0.3145	2.1008	0.6172
Prospective								
Male	23	48.94	34	41.46	1.00			
Female	24	51.06	48	58.54	1.3529	0.657	2.7931	0.4114
Gender/Time								
Male								
Prospective	23	43.4	34	29.57	1.00			
Premature	30	56.6	81	70.43	1.8265	0.9275	3.5934	0.0802
Female								
Prospective	18	85.71	24	75	1.00			
Premature	3	14.29	8	25	2	0.4983	10.1178	0.3524
Smoking								
No	55	74.32	107	72.79	1.00			
Yes	19	25.68	40	27.21	1.08	0.58	2.07	0.8077
Diabetes								
No	41	55.41	85	57.82	1.00			
Yes	33	44.59	62	42.18	0.91	0.52	1.60	0.7319
Hypertension								
No	16	21.62	36	24.49	1.00			
Yes	58	78.38	111	75.51	0.85	0.43	1.64	0.6354
Dyslipidemia								
No	30	40.54	59	40.14				
Yes	44	59.46	88	59.86	1.02	0.57	1.79	0.9539
Change in CRP								
No	24	34.29	47	36.15	1.00			
Yes	46	65.71	83	63.85	0.92	0.50	1.69	0.7923

ALN *								
0	5	7.35	8	5.88	1.00			
≤2	35	51.47	69	50.74	1.23	0.38	4.05	0.7307
>2	28	41.18	59	43.38	1.32	0.39	4.39	0.6541
TROPONIN I > 0.11 ng/ml								
No	13	58.82	18	56.62	1.00			
Yes	60	41.18	128	43.38	1.54	0.70	3.33	0.2752
CK-MB mass >5.6 ng/ml								
No	20	30.30	43	31.85	1.00			
Yes	46	69.70	92	68.15	0.93	0.49	1.75	0.8241
EF								
<50%	26	40.00	45	35.16	1.00			
≥50%	39	60.00	83	64.84	1.23	0.66	2.27	0.5099

Note: *C-reactive protein (CRP); Arteries Lesion Number (ALN); Ejection Fraction (EF); OR: Odds Ratio; CI: Confidence Interval; p: p-value, obtained through the logistic regression test. *Multinomial logistic regression.*

Conclusions

In summary, the NPY c.20T>C variant appears to contribute to the development of ACS. The NPY2R c.-1116A>G variant may contribute to the early development of ACS, and the NPY c.84G>A variant appears to contribute to the development of hypertension. In addition, the NPY c.20T>C is associated with a protective effect in ACS severity. This information contributes to a better understanding of the effect of NPY and NPY2R variants in the population under study. Further research with a larger sample size is necessary to confirm our results.

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Author contributions

Conception and design of the research: Soares F, Werkhauser RP, Moraes CNL, Martins DBG, Montenegro SML. Acquisition of data: Soares F, Araújo RM, Carvalho VDCV, Amorim EAS, Silva LCA, Montenegro ST, Neco HVPC. Analysis and interpretation of the data: Soares F, Araújo RM, Werkhauser RP, Bhaskar LVKS, Neco HVPC, Moraes CNL, Martins DBG, Montenegro SML. Statistical analysis: Diniz GT, Tashiro T. Obtaining financing: Montenegro SML. Writing of the manuscript: Soares F. Critical revision of the manuscript for intellectual content: Araújo RM, Werkhauser RP, Bhaskar LVKS, Carvalho VDCV, Amorim EAS, Silva LCA, Montenegro ST, Moraes CNL, Martins DBG, Montenegro SML.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Aggeu Magalhães Institute (FIOCRUZ/PE) under the protocol number CAAE: 03187512.2.0000.5202. All the procedures in this study were in accordance with the 1975 Helsinki Declaration, updated in 2013. Informed consent was obtained from all participants included in the study.

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