

SNP rs198389 (T-381 C) polymorphism in the B-type natriuretic peptide gene promoter in patients with atherosclerotic renovascular hypertension

Rafał Poręba¹, Karol Początek², Paweł Gać¹, Małgorzata Poręba³,
Marzena Gonerska³, Anna Jonkisz⁴, Arkadiusz Derkacz¹, Marta Negrusz-Kawecka²,
Małgorzata Sobieszkańska³, Witold Pilecki³, Andrzej Szuba¹, Ryszard Andrzejak¹

¹ Department of Internal Medicine, Occupational Diseases and Hypertension, Medical University, Wrocław, Poland

² Department of Cardiology, Medical University, Wrocław, Poland

³ Department of Pathophysiology, Medical University, Wrocław, Poland

⁴ Department of Forensic Medicine, Medical University, Wrocław, Poland

KEY WORDS

atherosclerosis,
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phism, renal artery
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renovascular
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ABSTRACT

INTRODUCTION Renovascular hypertension caused by renal artery stenosis (RAS) accounts for 3–5% of all cases of hypertension.

OBJECTIVES The aim of the study was to determine the association between SNP rs198389 (T-381 C) polymorphism in the B-type natriuretic peptide promoter (BNP) gene and the degree of RAS in patients with atherosclerotic renovascular hypertension.

PATIENTS AND METHODS Thirty-six patients scheduled for invasive diagnostic evaluation of atherosclerotic renovascular hypertension were enrolled in the study. Arteriography was performed through a femoral artery access. In order to identify SNP rs198389 (T-381 C) polymorphism in the BNP gene genotyping was performed using genomic DNA isolated from peripheral blood leukocytes. Amplified by polymerase chain reaction and purified product was used to minisequencing. Capillary electrophoresis was applied to separate and detect minisequencing products. The analysis enabled to discriminate TC heterozygotes, TT homozygotes, and CC homozygotes at position 381 of the BNP gene promoter.

RESULTS Patients homozygous for C allele occurred more frequently in patients with high-grade RAS in comparison with the moderate and mild stenosis groups. TT homozygotes were observed more frequently in mild stenosis patients compared to the moderate and high-grade stenosis groups. None of the patients who had mild RAS was homozygous for C allele and none of the patients who had severe RAS was homozygous for T allele.

CONCLUSIONS We demonstrated the association between SNP rs198389 (T-381 C) polymorphism in the BNP gene promoter and the degree of RAS in patients with atherosclerotic renovascular hypertension. It appears that subjects homozygous for C allele at position 381 of the BNP precursor gene promoter are more prone to develop atherosclerotic lesions in renal arteries.

INTRODUCTION Renovascular hypertension caused by renal artery stenosis (RAS) accounts for 3–5% of all cases of hypertension. It is considered that RAS can lead to glomerular ischemia and secondary blood pressure rise resulting from

the renin–angiotensin–aldosterone system activation. Disturbed autoregulatory mechanisms may also play a key role in the development of hypertension because of the renal blood flow reduction and the increase in intervascular fluid

Correspondence to:
Rafał Poręba, MD, PhD,
Katedra i Klinika Chorób
Wewnętrznych, Zawodowych
i Nadciśnienia Tętniczego,
Akademia Medyczna, ul. Pasteura 4,
50-367 Wrocław, Poland,
phone: + 48-71-784-25-20,
fax + 48-71-784-09-54, e-mail:
sogood@poczta.onet.pl
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volume.^{1,2} RAS in the elderly is usually associated with the presence of atherosclerotic lesions (atherosclerotic RAS – ARAS) and in the younger groups with fibromuscular dysplasia.^{3,4} The diagnosis of hypertension is crucial because it can identify patients with a potentially removable cause of the disease.

Currently, it is more and more commonly emphasized that evaluation of serum level of B-type natriuretic peptide (BNP) levels are important in predicting the effect of revascularization on kidney function. Initially increased serum BNP levels might be associated with polymorphism in the BNP gene precursor.

PATIENTS AND METHODS The study involved 36 patients, including 15 females and 21 males (mean age 57.8 ± 9.8 years), scheduled for invasive diagnostic evaluation of atherosclerotic renovascular hypertension on the basis of medical history, physical examination and non-invasive test results which help assess renal morphology and function. Recruitment of the patients for angiography was performed in accordance with the guidelines of the Polish Society of Hypertension Working Group and the experience of the clinical center. It was assumed that the following features are suggestive of atherosclerotic renovascular hypertension^{5,6}:

- 1 sudden onset of diastolic hypertension in patients aged ≥ 55 years
- 2 hypertension resistant to a combination therapy with at least 3 drugs
- 3 malignant hypertension
- 4 ineffective blood pressure control
- 5 recurrent episodes of pulmonary edema due to uncontrolled hypertension
- 6 moderate or severe hypertension in a patient with multilevel atherosclerosis
- 7 renal artery murmur
- 8 moderate or severe hypertension in a patient with renal failure of unknown etiology
- 9 urea and/or creatinine accumulation caused by angiotensin-converting enzyme or angiotensin II receptor blocker administration
- 10 asymmetry of the kidneys diagnosed by routine imaging examinations.

Arteriography was performed through a femoral artery incision. To visualize the renal arteries a nonselective injection of 50–60 ml nonionic and iso-osmolar contrast medium was injected to the abdominal aorta at the level of the renal arteries in standard projection with a lamp deviation to the left oblique (LAO 200). Some additional projections with selective contrast medium injection to the right and left renal artery were performed where required. The grade of vascular stenosis was assessed with digital imaging analysis. Lumen reduction $< 50\%$ was defined as mild RAS, the reduction between 50–69% was graded as moderate (grade 2) and stenosis higher than 70% was defined as severe (grade 3). The patients scheduled for percutaneous angioplasty intervention presented with severe RAS.

In order to identify SNP rs198389 (T-381 C) polymorphism in the BNP gene precursor, genotype analysis was preceded by genomic DNA extraction from peripheral blood leukocytes of the examined patients by applying the kit for extraction of DNA The QIAamp DNA Blood Mini Kit (QIAGEN GmbH company, Hilden, Germany). Polymerase chain reaction (PCR) was performed to detect SNP rs198389 (T-381 C) polymorphism using genomic DNA to amplify an appropriate DNA gene fragment. The QIAGEN Multiplex Kit and primers synthesized at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw were used. The sequence of primers was designed using the database of the National Center for Biotechnology Information GenBank Sequence Viewer Assai ID ss65835607. PCR was performed according to the protocol provided by the manufacturer. PCR products were then separated from unused primers and dideoxynucleotides in the amplification reaction using an enzymatic method.

The DNA fragment multiplied and purified as described was used to conduct minisequencing reaction using the SNaPshot Multiplex Kit by means of the ABI PRISM SNaPshot Multiplex Kit reagents from the Applied Biosystems Company. Capillary electrophoresis was used to separate and detect products of the minisequencing reaction and the ABI PRISM 3130 Genetic Analyser of the Applied Biosystems Company was employed. The reaction products were analyzed according the internal standard of Liz-120 using GeneMapperID v3.2 software. The analysis allowed to detect TC heterozygotes at position 381 of the BNP gene promoter among patients included in the study, that is patients with 2 different gene precursor alleles of BNP (with thymine nucleotide in the 1st allele and cytosine nucleotide in the 2nd one at position 381 of the gene promoter), TT homozygotes at position 381 of the BNP gene promoter (with thymine nucleotide in both alleles at position 381 of the gene promoter) and CC homozygotes at position 381 of the BNP gene promoter (with cytosine nucleotide in both alleles at position 381 of the gene promoter).

The study was approved by the Bioethics Committee at Wrocław Medical University.

Statistical analysis was carried out using Statistica PL 6.0 software (StatSoft, Poland), and means and standard deviations of the analyzed groups were calculated for continuous variables. The distribution of variables was verified by the W-Shapiro-Wilk test. The Kruskal-Wallis ANOVA test was performed for variables with non-normal distribution. Significant differences between means were analyzed by using the post hoc Newman-Keuls test. Categorical variables were presented as percentage. The χ^2 test was used to compare qualitative variables. A p-value < 0.05 was considered statistically significant.

RESULTS Hypertension in the patients was treated with combination therapy; none of the patients

TABLE 1 Clinical characteristics of patients divided into groups according to the grade of renal artery stenosis

	The grade of renal artery stenosis			p
	Group I <50% [n = 17]	Group II 50–69% [n = 10]	Group III ≥70% [n = 9]	
age ^a [years]	56.4 ± 10.8	58.1 ± 9.6	60.3 ± 8.2	NS
male ^b	10 (58.8%)	6 (60%)	5 (55.5%)	NS
SBP ^a [mmHg]	160.4 ± 5.8	165.1 ± 9.2	170.8 ± 4.3	I vs. III: p < 0.01
DBP ^a [mmHg]	89.5 ± 1.1	92.7 ± 2.4	99.4 ± 1.7	I vs. III: p < 0.01
hypertension duration [years]	5.6 ± 2.1	5.1 ± 1.7	6.3 ± 2.9	NS
creatinine ^a [μmol/l]	78.5 ± 25.3	80.1 ± 20.8	92.2 ± 29.5	I vs. II, III: p < 0.05
GFR ^a [ml/min]	109.4 ± 17.2	91.1 ± 15.4	82.2 ± 21.9	I vs. II, III: p < 0.05
BMI ^a [kg/m ²]	26.5 ± 5.2	27.8 ± 4.1	26.4 ± 3.9	NS
DM ^b	4 (23.5%)	2 (20%)	5 (55.5%)	I vs. II, III: p < 0.05
hyperlipidemia ^b	12 (70.6%)	7 (70%)	6 (66.7%)	NS
cigarette smoking ^b	11 (64.7%)	6 (60%)	6 (66.7%)	NS
stroke ^b	2 (11.8%)	1 (10%)	2 (22.2%)	NS
coronary artery disease ^b	9 (52.9%)	5 (50%)	5 (55.5%)	NS
peripheral artery disease ^b	4 (23.5%)	2 (20%)	4 (44.4%)	I vs. II, III: p < 0.05

a Values are given as mean ± standard deviation.

b Values are given as number (percentage).

Abbreviations: BMI – body mass index, DBP – diastolic blood pressure, DM – diabetes mellitus, GFR – glomerular filtration rate, NS – non significant, SBP – systolic blood pressure

received monotherapy. Twenty patients (55.5%) received 3 or more antihypertensive medications. Twenty-seven patients (75%) were treated with β-blockers, 22 (61.1%) with angiotensin-converting enzyme inhibitors, 16 (44.4%) with diuretics, 14 (38.9%) with calcium antagonists, and 5 (13.9%) with angiotensin II receptor blockers. In addition to antihypertensive agents, 18 patients (50%) received acetylsalicylic acid and 16 (44.4%) statins on a long-term basis.

All patients presented atherosclerotic lesions in the renal arteries. Mild RAS was observed in 17 patients (47.2%), moderate grade in 10 patients (27.8%) and severe in 9 patients (25%). The mild stenosis group had a statistically significant lower systolic and diastolic blood pressure in comparison to the group with severe stenosis (p < 0.01). Serum creatinine levels in patients with severe and moderate RAS were significantly higher compared to the patients with mild RAS (p < 0.05). Moreover, diabetes and peripheral artery disease occurred considerably more commonly in patients with severe RAS compared to the groups with moderate and mild RAS (p < 0.05). Clinical characteristics of the patient groups classified according to the grade of RAS are presented in [TABLE 1](#). Angiographically and clinically

successful percutaneous angioplasty with stent implantation was performed in 8 patients (88.9%) with severe RAS.

Twenty-one patients heterozygous for C allele (58.3%), 10 subjects homozygous for T allele (27.8%) and 5 (13.9%) subjects homozygous for C allele were identified by assessing SNP rs198389 (T-381 C) polymorphism in the BNP gene. The prevalence analysis of SNP rs198389 (T-381 C) polymorphism in the BNP gene in groups with various grades of RAS showed that CC homozygotes at position 381 of the BNP gene promoter occurred significantly more frequently in the severe stenosis group in comparison with moderate and mild stenosis patients (p < 0.05). However, patients with TT genotype at position 381 of the BNP gene promoter were observed substantially more commonly in the mild stenosis group compared to moderate and severe stenosis patients (p < 0.05). There was no patient homozygous for C allele among those who had mild RAS. None of the patients who had severe RAS had the TT genotype at position 381 of the BNP gene promoter ([TABLE 2](#)).

DISCUSSION The current study demonstrated an association between SNP rs198389 (T-381 C)

TABLE 2 SNP rs198389 (T-381 C) polymorphism in the B-type natriuretic peptide gene precursor in patient divided into the groups according to the grade of renal artery stenosis

	The grade of renal artery stenosis			p
	Group I <50% [n = 17]	Group II 50–69% [n = 10]	Group III ≥70% [n = 9]	
TC heterozygotes at position 381 of the BNP gene promoter ^a	8 (47.1%)	8 (80%)	5 (55.6%)	II vs. I and III: p < 0.05
TT homozygotes at position 381 of the BNP gene promoter ^a	9 (52.9%)	1 (10%)	0 (0%)	I vs. II and III: p < 0.05
CC homozygotes at position 381 of the BNP gene promoter ^a	0 (0%)	1 (10%)	4 (44.4%)	III vs. I and II: p < 0.05

^a Values given as number (percentage)

Abbreviations: BNP – B-type natriuretic peptide, other – see TABLE 1

polymorphism in the BNP gene precursor and the degree of RAS in patients with atherosclerotic renovascular hypertension. This relationship has already been recognized as one of the potential causes of renovascular hypertension.

Visualization of ARAS does not unequivocally prove renovascular hypertension. It is related to the fact that both prevalence of hypertension and atherosclerotic lesions of renal arteries increase with the patients' age. Usually, atherosclerotic lesions of renal arteries coexist with atherosclerosis of other vascular beds. They are identified in 16–28% of patients with atherosclerotic aortic lesions, in 22–45% of patients with peripheral artery disease of lower extremities and in 14–30% patients with ischemic heart disease.⁷

It is generally accepted that RAS seems to be an independent risk factor for adverse cardiovascular events. Therefore, search for diagnostic techniques that identify the patient group with a potentially reversible cause of hypertension is crucial. Causal treatment of renovascular hypertension using renal artery angioplasty leads to a decrease in blood pressure and above all prevents ischemic renal failure. Angiographical effectiveness of angioplasty with stent implantation is estimated to be 98%.⁸ The effectiveness of the therapeutic procedure used in the current study was slightly lower (88.9%). Available data indicate that despite highly beneficial short-term results, restenosis is still observed in 20–26% of patients.^{8,9} One of the approaches to improving long-term effectiveness of the procedures can be renal artery angioplasty optimization by using intravascular ultrasonography.¹⁰

Recently, the increased serum BNP level in patients with renovascular hypertension unrelated to heart failure and acute coronary syndromes has received considerable attention. It has been proven that an initially increased serum BNP level decreases after successful angioplasty procedure. Therefore, patients with initially increased serum BNP levels may represent a group where optimal antihypertensive effect can be achieved.^{11,12} Initially increased serum BNP levels are associated

with the polymorphism of a gene precursor. Therefore, it should be assumed that to identify polymorphism in the BNP gene precursor, genotype analysis should be performed. It allows an early identification of patients with high and low serum BNP levels.

It is assumed that CC homozygotes at position 381 of the BNP precursor gene promoter (with cytosine in both alleles at position 381 of the gene promoter) are characterized by high serum BNP levels. By contrast, patients homozygous for T allele at position 381 of the BNP gene promoter (with thymine in both alleles at position 381 of the gene promoter) have low serum BNP levels.^{13,14}

We demonstrated the association between SNP rs198389 (T-381 C) polymorphism in the BNP gene promoter and the degree of RAS in patients with renovascular hypertension caused by atherosclerosis. It appears that patients homozygous for C allele at position 381 of the BNP gene promoter are more prone to develop atherosclerotic lesions in renal arteries than TC heterozygotes and TT homozygotes.

We acknowledge limitations of the present study. First, the size of the patient group was relatively small especially when divided into 3 subgroups with a different grade of stenosis. Although level of statistical significance of the study has been reached, i.e. the study results were obtained from a homogenous groups, the number of patients was too small to extrapolate these findings for the whole population. Another limitation was the lack of measurement of serum BNP levels in the whole group and compared subgroups. We cannot address the issue of interrelations between clinical outcomes of angioplasty and the polymorphism tested. Analysis of these aspects is under way. We have focused primarily on what we considered to be of particular interest, namely the search for the association between SNP rs198389 (T-381 C) polymorphism in the BNP gene promoter and the degree of RAS in patients with atherosclerotic renovascular hypertension.

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Polimorfizm typu SNP rs198389 (T-381 C) w rejonie promotorowym genu peptydu natriuretycznego B u chorych na nadciśnienie naczyniowo-nerkowe na tle miażdżycy

Rafał Poręba¹, Karol Początek², Paweł Gać¹, Małgorzata Poręba³,
Marzena Gonerska³, Anna Jonkisz⁴, Arkadiusz Derkacz¹, Marta Negrusz-Kawecka²,
Małgorzata Sobieszczańska³, Witold Pilecki³, Andrzej Szuba¹, Ryszard Andrzejak¹

1 Katedra i Klinika Chorób Wewnętrznych, Zawodowych i Nadciśnienia Tętniczego, Akademia Medyczna, Wrocław

2 Katedra i Klinika Kardiologii, Akademia Medyczna, Wrocław

3 Katedra i Zakład Patofizjologii, Akademia Medyczna, Wrocław

4 Katedra i Zakład Medycyny Sądowej, Akademia Medyczna, Wrocław

SŁOWA KLUCZOWE

miażdżycza, nadciśnienie naczyniowo-nerkowe, polimorfizm genu, prekursor peptydu natriuretycznego B (BNP), zwężenie tętnicy nerkowej (RAS)

STRESZCZENIE

WPROWADZENIE Nadciśnienie naczyniowo-nerkowe, spowodowane zwężeniem tętnicy nerkowej, jest przyczyną 3–5% przypadków nadciśnienia tętniczego.

CELE Celem pracy było ustalenie zależności między polimorfizmem typu SNP rs198389 (T-381 C) w rejonie promotorowym genu peptydu natriuretycznego B (*B-type natriuretic peptide* – BNP) a stopniem zwężenia tętnicy nerkowej u chorych na nadciśnienie naczyniowo-nerkowe na tle miażdżycy.

PACJENCI I METODY Badaniami objęto 36 chorych zakwalifikowanych do diagnostyki inwazyjnej nadciśnienia naczyniowo-nerkowego na tle miażdżycy. Arteriografię wykonywano poprzez nakłucie tętnicy udowej. Analizę genotypu w celu określenia polimorfizmu typu SNP rs198389 (T-381 C) genu BNP przeprowadzono po wyizolowaniu genomowego DNA z leukocytów krwi obwodowej. Powielony w reakcji PCR i oczyszczony fragment wykorzystano do przeprowadzenia minisekwencjonowania. Do rozdzielenia i detekcji produktów minisekwencjonowania zastosowano elektroforezę kapilarną. Analiza pozwoliła wyodrębnić heterozygoty T/C, homozygoty T/T oraz homozygoty C/C w pozycji 381 promotora genu BNP.

WYNIKI Homozygoty C/C występowały znacznie częściej w grupie chorych z dużego stopnia zwężeniem tętnicy nerkowej w porównaniu z chorymi ze zwężeniem łagodnego i umiarkowanego stopnia. Homozygoty T/T występowały znacznie częściej w grupie chorych ze zwężeniem tętnicy nerkowej łagodnego stopnia w porównaniu z chorymi ze zwężeniem umiarkowanego i dużego stopnia. Żaden chory z łagodnym zwężeniem tętnicy nerkowej nie był homozygotą C/C i żaden chory ze zwężeniem dużego stopnia nie był homozygotą T/T.

WNIOSKI Wykazano zależność między polimorfizmem typu SNP rs198389 (T-381 C) genu prekursora BNP a stopniem zwężenia tętnicy nerkowej u chorych na nadciśnienie naczyniowo-nerkowe na tle miażdżycy. Wydaje się, że homozygoty C/C w pozycji 381 promotora genu BNP są bardziej narażone na rozwój zmian miażdżycowych w tętnicach nerkowych.

Adres do korespondencji:
dr med. Rafał Poręba,
Katedra i Klinika Chorób
Wewnętrznych, Zawodowych
i Nadciśnienia Tętniczego,
Akademia Medyczna,
ul. Pasteura 4, 50-367 Wrocław,
tel.: 071-784-25-20,
fax: 071-784-09-54, e-mail:
sogood@poczta.onet.pl
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