Association of *PPAR-γ2* and *β3-AR* Polymorphisms With Postmenopausal Hypertension

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The aim of this study was to test the association of peroxisome proliferator-activated receptor (*PPAR-γ2*) (Pro12Ala, C1431T) and *β3-AR* (Trp64Arg) polymorphisms with metabolic, nutritional, and blood pressure parameters in 271 postmenopausal women (151 hypertensive and 120 normotensive controls). The TaqMan genotyping assay and restriction fragment length polymorphism methods were used to determine the distributions of selected alleles and genotype frequencies. Nutritional status was determined by a bioimpedance method and dietary habits were assessed via 7-day dietary recall. The distribution of selected genotypes and allele frequencies did not differ between hypertensive women and normal controls after analysis by chi-square test. The postmenopausal hypertensive women were older and had higher body fat mass, serum glucose, and triglyceride levels. The cluster analysis showed that the hypertensive group with Pro12Pro genotype had highest pulse pressure and mean arterial pressure values when compared with Pro12Ala patients. In the logistic regression analysis, blood glucose (Pro12Ala polymorphism) and energy intake (C1431Tand T1431T polymorphisms) determined hypertension. *J Clin Hypertens (Greenwich).* 2015;17:549–556. © 2015 Wiley Periodicals, Inc.

Increased body weight, insulin resistance (IR), hyperglycemia, abnormal blood lipid profiles, oxidative stress, and hypertension (HTN) contribute to cardiovascular diseases.[1–4] With aging, a greater incidence of HTN and cardiovascular complications is widely observed in postmenopausal women compared with premenopausal women.[5,6] The hypertensive state is often associated with dyslipidemia,[7] obesity (nearly 40% of hypertensive patients are obese),[8] and IR.[9] Moreover, blood pressure (BP) in women presents specific hemodynamic characteristics, and, in the postmenopausal age, the role of pulse pressure (PP) predominates over that of mean arterial pressure (MAP) in the mechanism of high BP. The stiffness of the large elastic arteries in cardiothoracic (central) circulation increases with age[10] and it results in progressive age-associated elevations in SBP and PP. These changes accelerate after the age of 50 and are much greater in women than in men.[5,6]

Studies have shown that *PPAR-γ2* polymorphisms (Pro12Ala, C14131T) and β-adrenergic receptor gene polymorphisms (*β3-AR* Trp64Arg) may have an important impact on adipose tissue,[11] metabolic parameters,[12,13] and inflammation in the vasculature, and through their influence determine high BP.[14,15] Some data have indicated that Pro12Pro normoglycemic homozygotes had significantly higher incidence of HTN[16] and higher diastolic BP (DBP)[17] when compared with Ala12 carriers. However, the association between the Pro12Ala variants and arterial BP remains controversial and most other studies could not demonstrate such an association,[18,19] while some even indicated that Ala12 carriers had higher BP.[20] Besides this, the polymorphisms have been mostly studied separately (not in constellation) in patients with diabetes mellitus or obesity who were treated with hypoglycemic and hypolipidemic medications. To our knowledge, this is the first study to analyze the associations of three coexisting polymorphisms with HTN, metabolic state, and nutritional habits in postmenopausal women without hypoglycemic and hypolipidemic treatment.

MATERIAL AND METHODS

Study Population

Data based on clinical history and anthropometrical parameters were selected from 1423 women aged 49 to 75 years who underwent standard health checkups at a metabolic outpatient clinic. The gynecological interview and hormonal profile including the measurement of follicle-stimulating hormone (FSH, foliculotropic hormone) confirmed the postmenopausal period. From this group, only women without diseases or treatment that may influence glucose and lipid status were included in this study. Patients with cardiovascular diseases (with the exception of HTN), endocrinological disorders (eg, diabetes mellitus), renal or liver insufficiency, acute infection and hematologic diseases, previously diagnosed or treated dyslipidemia, were excluded from the study. Women using hormonal replacement therapy,

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hypolipidemic treatment, or medications that affect glucose metabolism (eg, hypoglycemic drugs, glucocorticoids) were also eliminated from the study. Thus, we selected 271 postmenopausal women to undergo biochemical, nutritional, and genetic evaluation. The selection was random because *PPAR-γ* status was unknown at the time of recruitment. All patients provided written informed consent on documents approved by the local Bioethical Commission of Poznan Medical University, Poland, nr 792/09, and followed the guidelines proposed by the Declaration of Helsinki. Written informed consent was obtained from each patient before commencement of the study.

Clinical Data

Clinical data, including anthropometric and nutritional status, biochemical assessment, and history of HTN were obtained from each patient. Anthropometric measurement included weight and height measured in underwear using the SECA scale. Waist circumference was determined at the narrowest point between the costal margin and iliac crest, and the hip circumference was measured at the widest point over the buttocks. Body mass index was calculated as weight/height squared (kg/m^2) and waist to hip ratio (WHR) as the proportion of waist to hip circumferences.[21] A bioimpedance analyzer (Bodystat 1500; Bodystat Ltd, Isle of Man, United Kingdom) was used to assess fat content as a proportion of total body mass. The bioimpedance analysis was performed with a single-frequency (50 kHz) device.

The antecedent of HTN was obtained retrospectively by checking medical records. BP was measured in triplicate using the patient's nondominant arm following a 10-minute rest using a standard mercury sphygmomanometer during the single visit (the mean of three measurements of systolic BP [SBP] and DBP was calculated). It was recorded at the upright seated position between 7 AM and 11 AM after an overnight fast in strict accordance with guidelines of the European Society of Hypertension's Working Group on Blood Pressure Monitoring.[22] The diagnosis of HTN was given if SBP exceeded 140 mmHg and/or DBP was higher than 90 mm Hg. MAP was approximated using the equation: MAP=[(SBP+2×DBP)]/3. PP was calculated as the difference between SBP and DBP.[23]

Biological samples were collected in the morning (7 AM) after a 12-hour fast and after a minimum of 48 hours since the last period of physical exercise. Participants were in a seated position and had rested for 5 minutes. Venous blood samples were collected in EDTA-containing tubes. The serum samples were taken from clotted (15 minutes, RT) and centrifuged (15 minutes, 3000 *g*) blood drawn by venous puncture following a 12-hour overnight fast. Uric acid and glucose and lipid profile (total cholesterol [TC], high-density lipoprotein [HDL] and low-density lipoprotein [LDL], triglycerides [TGs]) were determined with enzymatic colorimetric assays (Cobas Integra 400 Plus; Roche Diagnostics, Indianapolis, IN). Samples were immediately centrifuged and serum was separated and directly used for the assay. The serum level of LDL was calculated using the formula of Friedewald and colleagues.[24] Serum lipoprotein (a) levels were measured by an immunoenzymatic assay (Cormay, Siedlce, Poland). FSH serum levels were measured via specific chemiluminescence assays from Roche Diagnostic. Plasma insulin levels were determined by means of an enzymatic immunoassay [Cobas Integra 400 Plus; Roche Diagnostics]. The original homeostatic model assessment model of insulin resistance (HOMA1-IR) was calculated as described by Matthews and colleagues[25]: HOMA1-IR = [fasting insulin (μu/mL) × fasting glucose(mM)]/22.5

Nutritional Evaluation

During the study, all patients were on a normal diet (without any dietary modifications). Food intake was assessed by 24-hour recall during 7 days.[26] The results of the questionnaire were analyzed using both quantitative and qualitative analysis of the patients' daily diets[27] using computer databases for Microsoft Access 2000 (Microsoft Corporation, Redmond, WA).[28] The food intake recommendations of the National Institute of Food and Nutrition in Warsaw, Poland, were taken into consideration to determine the degree to which the Recommended Dietary Allowances were met.[29]

Genotyping

Genomic DNA was isolated from venous blood samples according to the manufacturer's protocol (Gentra Puregene Blood Kit; QIAGEN, Venlo, Limburg). Genotypes of the Pro12Ala (rs1801282) and Trp64Arg polymorphism (rs4994) were determined by a TaqMan genotyping assay (Life Technologies, Carlsbad, CA). As a quality-control measure, negative controls and approximately 5% of samples were genotyped in duplicate to check genotyping accuracy. The controls for each of the genotypes of both single nucleotide polymorphisms were run in parallel. An allelic discrimination assay was performed on an ABI7900HT or on CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc, Hercules, CA). C1431T (rs3856806) genotyping was performed using PCR-restriction fragment length polymorphism (PCR-RFLP) analysis. Eco72I cleaves the PCR product from wild-type DNA to generate fragments of 127bp and 43bp, but does not cut products containing the variant allele. PCR-digests were analyzed on 2.5% agarose gels. The determination of the ADRB3 variant was performed using the PCR method as described by Sivenius and colleagues[30] The genotypes were determined as Trp64Trp, Trp64Arg, and Arg64Arg without prior knowledge of the patients' status.

Linkage Disequilibrium Block Determination and Haplotype Construction

The genotype data were used to construct the haplotypes between the two polymorphisms by using Haploview 4.2 software (Broad Institute, Cambridge, MA) to evaluate linkage disequilibrium (LD). LD between the

TABLE I. Genotype and Allele Frequencies of the Pro12Ala and C1431/X *PPARγ2* and Trp 64Arg of β-Adrenergic Receptor Gene Polymorphisms According to Normotensive (RR <140/90 mm Hg) and Hypertensive State (RR ≥140/90 mm Hg)

Analyzed Genotypes	Hypertensive Women (n=151)	Normotensive Women (n=120)	Whole Group of Postmenopausal Women (N=271)
Genotype			
Pro12Pro	101 (66.89)	84 (70.0)	185 (68.27)
Pro12Ala	44 (29.14)	32 (26.67)	76 (28.04)
Ala12Ala	6 (3.97)	4 (3.33)	10 (3.69)
Chi-square value (P value)	0.3149 (.5698)		
Allele frequency			
Pro	246 (0.815)	200 (0.833)	446 (0.823)
Ala	56 (0.185)	40 (0.167)	96 (0.177)
Chi-square value (P value)	0.3230 (.8542)		
OR (95% CI)	1.1382 (0.7282–1.7790)		
Genotype			
C1431C	107 (70.86)	91 (75.83)	198 (73.07)
C1431T	39 (25.83)	27 (22.50)	66 (24.35)
T1431T	5 (3.31)	2 (1.67)	7 (2.58)
Chi-square value (P value)	1.2304 (.5405)		
Allele frequency			
C1431	253 (0.838)	209 (0.871)	462 (0.852)
T1431	49 (0.162)	31 (0.129)	80 (0.148)
Chi-square value (P value)	1.1634 (.2808)		
OR (95% CI)	1.306 (0.8034–2.1222)		
Genotype			
Trp64Trp	122 (80.79)	98 (81.67)	220 (81.18)
Trp64Arg	27 (17.88)	20 (16.67)	47 (17.34)
Arg64Arg	2 (1.32)	2 (1.67)	4 (1.28)
Chi-square value (P value)	0.1161 (.9435)		
Allele frequency			
Trp	271 (0.897)	216 (0.900)	487 (0.899)
Arg	31 (0.103)	24 (0.100)	55 (0.101)
Chi-square value (P value)	0.0102 (.9192)		
OR (95% CI)	0.9713 (0.5536–1.7040)		

Abbreviations: CI, confidence interval; OR, odds ratio; PPARγ2, peroxisome proliferator-activated receptor γ2. Data are expressed as number (percentage) for genotypes and number (frequency) for alleles.

single nucleotide polymorphisms used in haplotype analysis was measured using a pairwise D' statistic. The structure of the LD block was examined with the method proposed by Gabriel and colleagues[31] using the 80% confidence bounds of D'. The haplotype frequencies were calculated based on the maximum likelihood method with Haploview 4.2 software. Finally, the associations between haplotypes and obesity status were checked. Specific haplotype frequencies were compared among lean and obese women (chi-square test).

Statistical Analysis

The Shapiro-Wilk test was used in order to determine whether the continuous variables were normally distributed. Continuous data were shown as mean±standard deviation. The hypothesis that the differences between analyzed anthropometric and metabolic and factors in the analyzed groups were significant was tested by Mann-Whitney U test. Because the number of Ala12Ala homozygotes was small (one lean woman and nine obese women), this was collapsed with Pro12Ala heterozygotes and compared with Pro12Pro homozygotes for all analyses. Similarly, C1431T heterozygotes and T1431T homozygotes were collapsed together as well as Trp54Arg heterozygotes and Arg64Arg homozygotes. Allele frequencies were estimated using the gene-counting method, and an exact test was performed to identify departures from Hardy-Weinberg proportions (Court lab – HW calculator.xls).

Data mining of the study results was also performed by generalized k-means clustering. The k-means algorithm gains results into k clusters, where k is provided as an input parameter (k<N, where N is the size of the study group). First the algorithm arbitrarily selects k points as the initial cluster centers ("means"). Each point in the dataset is assigned to the closed cluster based on the Euclidean distance between each point and each cluster center. Each cluster center is then recom-

puted as the average of the points in that cluster. The procedure is repeated until the clusters converge, ie, no observations change clusters or the changes do not make a difference in the definition of the clusters. The statistical analysis was performed using STATISTICA for Windows, version 10.0 (StatSoft, Inc, Tulsa, OK).

Backward stepwise logistic regression was performed to determine the risk factors of HN. In each model, the odds ratio (OR) for each independent variable with a 95% confidence interval (CI) were determined. A *P* value below .05 was regarded as statistically significant. The statistical analyses were performed with STATISTICA 10 (including STATISTICA Medical Package 2.0; StatSoft, Inc, 2014 software) and SPSS 22 (IBM, Inc, Chicago, IL).

RESULTS

The differences between genotypes' distribution and allele frequencies of the Pro12Ala, C1431T, and Trp64-Arg polymorphisms between hypertensive and normotensive groups (calculated by chi-square test) were statistically insignificant (Table I). The observed genotype frequencies of polymorphisms were all in agreement with the Hardy-Weinberg equilibrium in the control patients.

The serum level of FSH, insulin, glucose, HDL, TGs, uric acid, and lipoprotein(a) were within the normal range, while TC and LDL levels exceeded the recommended levels in both groups (TC=190 mg/dL and LDL=130 mg/dL) (Table II). The hypertensive women were older (*P*=.0242) and had higher body fat mass (*P*=.0034), serum glucose (*P*=.0046), TG level (*P*=.0073), and HOMA-IR score (*P*=.0192). The nutritional analysis revealed higher-than-recommended energy intake, protein, and fat in both groups. The intake of sodium was high in both groups (an adequate intake [AI=1500 mg/d) and potassium supply did not achieve recommended values (AI=4700 mg/d).

The selected variants of all three analyzed polymorphisms (Pro12Ala, C1431T, and Trp64Arg) did not differ significantly in the whole group (Table III). However, the analysis of the SBP and DBP, as well as PP and MAP, differed within the same variant of selected

TABLE II. Anthropometric, Metabolic, and Nutritional Characteristics of Normotensive and Hypertensive Postmenopausal Women

Analysed Parameters	Hypertension (n=151), Mean±SD	Normal Blood Pressure (n=120), Mean±SD	P Value
Systolic blood pressure, mm Hg	157.29±15.26	121.02±10.86	**.00001**
Diastolic blood pressure, mm Hg	96.37±11.60	77.42±6.81	**.00001**
PP, mm Hg	60.92±10.92	43.60±9.20	**.00001**
MAP, mm Hg	116.67±11.87	91.95±7.17	**.00001**
Age, y	59.88±5.07	58.59±5.86	**.0242**
Body mass, kg	77.33±15.25	75.15±17.58	.1581
Height, cm	160.43±5.71	161.58±5.90	.1991
Triceps skinfold, mm	19.99±5.92	19.39±6.33	.4348
Body fat mass, %	44.46±6.79	42.22±6.44	**.0034**
BMI, kg/m^2	29.86±5.94	28.87±6.75	.2094
FSH, mIU/mL	69.43±26.96	70.87±25.63	.4958
Insulin, mU/mL	10.00±6.26	9.29±8.83	.0518
Glucose, mg/dL	98.36±14.75	94.28±11.53	**.0046**
TC, mg/dL	235.34±41.12	225.26±40.22	.0562
HDL, mg/dL	64.15±15.26	63.79±14.03	.9577
TG, mg/dL	124.12±55.28	109.14±51.06	**.0073**
LDL, mg/dL	146.33±37.18	139.69±35.96	.1561
UA, mg/dL	4.89±1.05	4.74±1.14	.1339
Lp, g/L	0.26±0.39	0.17±0.24	.0648
HOMA-IR	2.50±1.73	2.23±2.27	**.0192**
Energy, kcal	2042.36±555.32	2058.52±563.33	.8472
Protein–% energy	16.27±3.09	16.35±3.54	.6616
Fat–% energy	33.93±5.06	34.17±5.60	.8168
Carbohydrates–% energy	50.64±6.22	50.34±7.33	.9471
Sodium, mg/d	2278.76±848.05	2015.54±86.11	.0808
Potassium, mg/d	3596.98±952.22	3286.94±933.21	.0814
Calcium, mg/d	700.60±270.42	756.58±245.21	**.0385**
Phosphorus, mg/d	1345.86±395.52	1363.11±329.01	.3552
Magnesium, mg/d	332.47±97.90	328.57±86.27	.8338
Dietary fiber, mg/d	22.57±7.50	22.18±7.18	.8742

Abbreviations: BMI, body mass index; FSH, follicle-stimulating hormone; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; Lp, lipoprotein (a); MAP, mean arterial pressure; PP, pulse pressure; SD, standard deviation; TC, total cholesterol; UA, uric acid. Bold values indicate significance.

TABLE III. Differences in SBP, DBP, PP, and MAP in Postmenopausal Women According to Genotype Distribution

Analyzed Group	Analyzed Polymorohism	No.	SBP, Mean±SD	DBP, Mean±SD	PP, Mean±SD	MAP
Whole group	Pro12Pro	185	141.67±22.28	88.08±13.54	53.59±12.83	105.95±15.70
	Pro12Ala	76	140.01±22.91	86.91±13.73	53.11±14.35	104.61±15.96
	Ala12Ala	10	142.20±26.93	94.50±15.62	47.70±11.83	110.40±19.32
	All patients	271	141.21±22.57	87.99±13.69	53.22±13.25	105.73±15.89
Statistical significance			ns	ns	ns	ns
Whole group	C1431C	198	139.77±21.68	87.45±13.04	52.32±12.78	104.89±15.29
	C1431T	66	144.50±23.73	88.55±14.31	55.95±14.48	107.20±16.66
	T1431T	7	151.57±31.26	97.43±19.26	54.14±16.14	115.47±22.70
	All patients	271	141.23±22.52	87.98±13.57	53.25±13.33	105.78±15.89
Statistical significance			ns	ns	ns	ns
Whole group	Trp64Trp	220	141.36±22.14	88.07±13.64	53.29±12.72	105.83±15.85
	Trp64Arg	47	141.26±23.94	87.60±13.51	53.66±15.55	105.48±16.09
	Arg64Arg	4	133.75±31.69	87.25±14.13	46.50±20.53	102.75±19.34
	All patients	271	141.23±22.52	87.98±13.57	53.25±13.33	105.73±15.89
Statistical significance			ns	ns	ns	ns
Hypertension	**Pro12Pro**	**101**	**158.22±14.97**	**96.75±11.36**	**61.47±10.06**	**122.88±10.27**
	Pro12Ala	**44**	**155.30±15.62**	**94.75±12.12**	**60.55±12.79**	**120.74±10.99**
	Ala12Ala	**6**	**158.67±20.59**	**104.83±9.99**	**53.83±10.65**	**126.00±12.26**
Normal blood pressure	**Pro12Pro**	**84**	**122.28±10.02**	**77.83±6.94**	**44.45±8.86**	**92.54±6.96**
	Pro12Ala	**32**	**119.00±12.01**	**76.13±6.70**	**42.88±9.20**	**90.42±7.69**
	Ala12Ala	4	**117.50±10.66**	79.00±5.60	**38.50±6.56**	**91.83±7.02**
All patients		271	141.33±22.45	88.01±13.63	53.31±13.20	105.73±15.89
Ss			P<.001	P<.001	P<.001 (P<.0001[a])	P<.001
Hypertension	**C1431C**	**107**	**155.72±14.43**	**95.71±11.35**	**60.01±10.30**	**121.61±9.88**
	C1431T	**39**	**160.54±15.85**	**96.82±11.81**	**63.72±11.56**	**123.23±11.71**
	T1431T	**5**	**165.60±23.84**	**107.00±12.35**	**58.60±16.91**	**131.50±11.79**
Normal blood pressure	**C1431C**	**91**	**121.02±11.21**	**77.75±6.64**	**43.27±8.91**	**92.17±7.32**
	C1431T	**27**	**121.33±9.86**	**76.59±7.53**	**44.74±10.41**	**91.51±6.79**
	T1431T	**2**	**116.50±12.02**	**73.50±3.54**	**43.00±8.49**	**87.83±6.36**
All patients		271	141.23±22.52	87.98±13.57	53.25±13.33	**105.73±15.89**
Ss			P<.001	P<.001	P<.001	P<.001
Hypertension	**Trp64Trp**	**122**	**157.07±15.43**	**96.57±11.50**	**60.50±10.81**	**122.16±10.67**
	Trp64Arg	**27**	**158.15±15.19**	**95.30±12.51**	**62.85±11.78**	**124.44±10.20**
	Arg64Arg	**2**	**159.00±9.90**	**98.50±9.19**	**60.50±0.71**	**118.67±9.43**
Normal blood pressure	**Trp64Trp**	**98**	**121.80±10.60**	**77.49±7.14**	**44.31±8.51**	**92.26±7.43**
	Trp64Arg	**20**	**118.45±10.92**	**77.20±5.43**	**41.25±10.64**	**90.95±5.85**
	Arg64Arg	**2**	**108.50±19.09**	**76.00±2.83**	**32.50±21.92**	**88.83±4.48**
All patients		271	141.23±22.52	87.98±13.57	53.25±13.33	105.73±15.89
Ss			P<.001	P<.001	P<.001 (P<.0001[b])	*P*<.001 (P<.0001[b])

Abbreviations: DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SD, standard deviation; SDP, systolic blood pressure; Ss, statistical significance between normotensive and hypertensive patients with the same variant of polymorphism. [a]*P*<.0001 between normotensive patients with Ala12Ala and others Pro12Ala polymorphisms of normotensive and hypertensive women. [b]*P*<.0001 between normotensive patients with Arg64Arg and others Trp64Arg polymorphisms of normotensive and hypertensive women.

genotypes and were higher in hypertensive patients. Women with Ala12Ala genotype or Arg64Arg revealed lower PP compared with other normotensive and hypertensive patients, but the number of women with such polymorphism was small. The differences between metabolic and nutritional parameters in analyzed genotypes were statistically insignificant (data not shown).

The cluster analysis showed the lack of differences between metabolic parameters such as lipid profile, uric acid, and lipoprotein level); however, significant differences in PP and MAP values was observed between group 1 (hypertensive women with Pro12Pro genotype) and other groups (group 2: women normotensive and hypertensive with Ala12/X polymorphism; group 3: normotensive patients with Pro12Pro; and group 4: unclassified group (in this group, the analyzed patients considerably differed from data evaluated in generalized *k*-means clustering) (Table IV).

In the hypertensive group, a blood glucose level >95 mg/dL (Pro12Ala genotype) and energy intake >2002 kcal/d in C1431T and >1563 kcal/d in T1431T genotype were identified as prognostic factors of HTN

TABLE IV. Cluster Analysis of Association between, Genotype Distribution, Selected Metabolic Parameters and the Blood Pressure Value

Analysed Polymorphisms in Normo- and Hypertensive Women	Group[a] (Cluster) Number	TC [mg/dL] X±SD	HDL [mg/dL] X±SD	TG [mg/dL] X±SD	LDL [mg/dL] X±SD	UA [mg/dL] X±SD	Lp [g/L] X±SD	PP[1,2,3] X±SD	MAP[1,2,3] X±SD
Hypertensive women with Pro12Pro polymorphism	1 (n=96)	238.00±43.20	64.49±15.62	123.49±49.59	148.76±39.12	4.85±1.08	0.24±0.34	61.32±10.17	117.53±11.54
Women with Ala12/X polymorphism	2 (n=73)	230.14±39.69	64.40±15.38	120.53±63.32	141.47±4.10	4.92±1.13	0.24±0.40	52.48±14.18	104.75±16.25
Normotensive women with Pro12Pro polymorphism	3 (n=78)	222.70±40.54	62.95±13.09	109.38±52.15	138.11±37.60	4.71±1.10	0.18±0.27	44.44±9.05	92.85±6.89
Unclassified	4 (n=9)	223.12±27.63	60.86±12.82	139.74±44.09	134.33±24.77	5.20±0.68	0.23±0.17	47.78±12.55	111.59±20.10
	Whole group	230.58±41.25	63.87±14.67	118.92±54.61	142.93±36.98	4.84±1.09	0.22±0.33	53.18±13.21*	106.16±15.99**
Statistical significance		ns	ns	ns	ns	ns	ns	**	**

Abbreviations: HA, hypertensive patients; HDL, high density lipoproteins; LDL, low density lipoproteins; Lp, lipoprotein; MAP, mean arterial pressure; n, number of analysed subjects; ns, statistically not significant; PP, pulse pressure; SD, standard deviation; TC, total cholesterol; UA, uric acid; X, mean value. **Indicates P value<.0001 (groups with very significant differences are shown in superscript arabic numerals). [a]Group 4 does not differ from group 1, 2, 3 because of small number of analysed subjects in this group.

(Table V). The value of normal BP depended on age in women with C1431T polymorphisms (C1431C, T1431T, and C1431T).

DISCUSSION

This study has shown that HTN is a multifactorial disease and metabolic (glucose and lipid parameters), nutritional (energy intake), and genetic (Pro12Pro polymorphism) factors contribute to a hypertensive state. Even though were no differences between genotype distribution and allele frequencies of analyzed polymorphisms between hypertensive and normotensive women (Table I), we found some hemodynamic, anthropometric, and metabolic differences between the analyzed groups (Table II). Moreover, further analysis showed that some genetic factors may predispose to higher BP (Table IV and V). In the group of hypertensive patients (Table II), SBP and DBP were higher than that of the normotensive patients (36 mm Hg and 16 mm Hg, respectively) and, thus, may contribute to increased mortality from cardiac pathology.[32–36] Moreover, BP presents particular hemodynamic characteristics in women and refers to stroke volume, arterial stiffness, and wave reflections. The last two parameters impinge on PP in large arteries and, in postmenopausal women, the role of PP predominates over that of MAP in the mechanism of high BP.[10,37,38] In both analyzed groups, PP was higher and exceeded about 43 mm Hg in normotensive and 60 mm Hg in hypertensive patients (normally, this value should not cross the value of 30–40 mm Hg). Such wide PP is a known risk factor associated with higher mortality and higher risk of myocardial infarction.[36]

The analyzed women with HTN were older, which confirms the fact that BP increases with age.[2] We observed high body mass index values in both normotensive and hypertensive groups, which was about 29 kg/m^2 and indicated overweight (Table II). However, hypertensive women had higher body fat mass, which was centrally distributed. Visceral fat induces the development of HTN through increased activity of adipose tissue renin-angiotensin-aldosterone system, sympathetic stimulation, and mechanisms related to IR.[39] In such a state, compensatory hyperinsulinemia is associated with elevated BP.[40] Fortunately, blood glucose and TG levels and HOMA-IR score were within the normal range in both groups in this study, but these parameters were significantly higher in hypertensive patients. In normotensive and hypertensive women, hypercholesterolemia was diagnosed for the first time (elevated LDL level >130 mg/dL) and hypolipidemic treatments were not yet implemented (which was determined by exclusion criteria). Many studies proved that HTN and hypercholesterolemia act synergistically with cardiovascular risk factors.[9,41] However, dietary treatment of these disorders can make a substantial contribution to reducing hyperlipidemia, improving glycemic control, and treating HTN. Unfortunately, energy intake as well as energy percentage from protein and fat were higher than the

TABLE V. Factors Associated With Level of Blood Pressure Evaluated by Multiple Logistic Regression Analysis

Blood Pressure	Genotype	Constant Parameter	Mean±SD	Crude OR, SE	Adjusted OR	95% CI for Adjusted OR	*P* Value
Hypertension	Pro12Ala	Glucose, mg/dL	95.20±11.74	4.472	4.982	0.001–29.56	**.026**
	C1431T	Energy, kcal/d	2002.95±526.52	3.256	5.211	1.051–11.504	**.022**
	T1431T	Energy, kcal/d	1563.43±393.60	3.255	5.212	0.001–8.050	**.022**
Normal blood pressure	C1431C	Age, y	60.12±5.30	0.161	3.772	0.533–5.003	**.049**
	C1431T	Age, y	59.12±5.30	0.164	3.620	0.732–6.531	**.049**
	T1431T	Age, y	59.60±3.46	0.160	3.773	0.097–3.875	**.049**

Abbreviations: CI, confidence interval; OR, odds ratio; SD, standard deviation; SE, standard error. Bold values indicate significance.

recommended quantity in both groups (proper intake of energy should equal 1800 kcal/d, with energy from proteins <15% of energy intake and that from fats <30% of energy consumption).[21,29] In hypertensive women, we observed higher intake of sodium and lower intake of potassium (the differences were close to statistical significance). High sodium intake increases serum sodium and risk of HTN (hypernatremia causes the reduction of renal plasma flow and calcium excretion).[42] The low potassium supply may contribute to elevated BP.[29]

Genetic analysis showed that the differences between BP, PP, and MAP values do not depend on genotype distribution but are the result of HTN (Table III). It can be speculated that this fact may be determined by the lack of essential metabolic disorders (analyzed women were characterized by proper glycemic state and moderate hypercholesterolemia), while in other studies HTN has been modestly associated with IR and diabetes mellitus.[43] However, the cluster analysis revealed that the hypertensive women with Pro12Pro genotype had the highest values of PP and MAP when compared with the women with Ala12/X or normotensive women with Pro12Pro genotype (Table IV). Thus, the presence of Pro12Pro may be a risk factor for HTN in postmenopausal women. Similar data have shown that Pro12Pro homozygotes without diabetes mellitus revealed significantly higher incidence of HTN[16] and higher DBP[17] when compared with Ala12 carriers.

The value of HTN in a multivariate analysis was significantly related to the level of blood glucose in patients with Pro12Ala polymorphism and energy intake in patients with C1431T and T1431T genotype (Table V). Thus, our findings show that the polymorphisms of the *PPAR*-γ2 gene are associated with postmenopausal HTN. They are also involved in the complex pathogenesis of this multifactorial disease and have a synergic effect with other risk factors that can be modified by changes to diet (energy intake and glucose level). We suggest that genetic screening involving these polymorphisms could be useful for selecting higher-risk patients with HTN.

Study Strengths and Limitations

As with most studies involving human participants, our examination presented the following limitations: (1) patients were selected from an outpatient clinic, specializing in metabolic disorders, rather than chosen randomly from the surrounding community; (2) causality cannot be demonstrate, only association between variables; (3) adjustment for other potential confounders, such as socioeconomic status, physical activity, and family history of obesity was not performed; therefore, these data should be incorporated into future studies.

The strengths of the study were that: (1) all participants were strictly selected (271 patients were selected from the group of 1423 women); (2) women using hormonal replacement therapy and hypoglycemic and hypolipidemic treatment were excluded to eliminate all factors, which may influence gene expression (eg, thiazolidinediones); (3) it was a multidisciplinary study including anthropometrical, biochemical, nutritional, and genetic evaluation.

CONCLUSIONS

The observed differences in anthropometrical, nutritional, and BP hemodynamic parameters were determined by the hypertensive state. The presence of Pro12Pro genotype was characterized by higher PP and MAP values in cluster analysis. The value of blood glucose in women with Pro12Ala polymorphism and energy intake in patients with C1431T polymorphisms are the prognostic factors of HTN. The results support the assumption that increased blood glucose concentration and high energy intake could be indirect modifiable indicators of this disease.

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