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Association Analysis of 24-h Blood Pressure Records with I/D ACE Gene Polymorphism and ABO Blood Group System

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Summary

The purpose of this study was to analyze the association between 24-h blood pressure parameters, insertion/deletion polymorphism of the angiotensin I converting enzyme gene and the ABO blood group system in a sample of the general Czech population. Fourteen parameters describing the 24-h blood pressure readings were obtained by analyzing blood pressure records in 243 volunteers, 119 men and 124 women. These parameters were adjusted for sex and body mass index (BMI) by means of multiple regression test. All subjects were genotyped for the insertion/deletion polymorphism of angiotensin I converting enzyme (I/D ACE) gene and routinely examined for the blood group phenotype in the ABO system. An association was found between the interaction of I/D ACE gene polymorphism and ABO blood group system on the one hand and mean values of systolic ($p=0.016$) or mean arterial ($p=0.027$) blood pressures and the phase shift of 24-h BP rhythm ($p=0.036$) on the other hand. Among three I/D ACE variants the DD genotype was associated with the highest values of mean blood pressure in blood group A and AB carriers. The same genotype was associated with the lowest blood pressures in blood group B and O carriers. In subjects with the DD genotype, the earlier daily position of the maximum of 24-h BP rhythm was found in blood group B, AB and O carriers. On the contrary, blood group A was associated with the latest position of maximum of the 24-h BP rhythm in the DD genotypes.

Key words

Circadian blood pressure rhythm • I/D ACE polymorphism • ABO blood group system • Sex • Body mass index

Introduction

Chronobiological analysis of 24-h blood pressure records by approximating 24-h and 12-h rhythmic components had made it possible to define fourteen parameters from the 24-h blood pressure readings. Thus, the possibility of a more detailed analysis on the influence of genetic determinants in blood pressure control is being introduced.

The gene coding for angiotensin I converting enzyme (ACE) belongs to the most intensively studied candidate genes for essential hypertension. The insertion/deletion polymorphism of angiotensin-I converting enzyme in intron 16 is based on the presence or absence of the *Alu* repetitive sequence. This has been found to be associated with serum ACE levels (Rigat *et al.* 1990). An independent effect of the blood group system ABO on ACE serum activity was described

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recently by our group (Cídl *et al.* 1996). However, the functional significance of ACE levels in the serum is not yet completely understood in man. An enhanced pressor response to angiotensin I in normotensive men with DD genotype for I/D ACE polymorphism was shown, probably as a consequence of the generation of increased levels of angiotensin II (Ueda *et al.* 1995). The DD genotype was associated with significantly higher (p=0.003) wall thickness of the common carotid artery (Castellano *et al.* 1995), without simultaneous association of I/D ACE polymorphism with blood pressure values, according to 24-h blood pressure recordings.

In our study, the association of blood pressure parameters with I/D ACE gene polymorphism and the ABO blood group system phenotype was studied in healthy Caucasian subjects after their blood pressure parameters had been adjusted for sex and BMI.

Methods

A sample of the general population of the city of Brno (400 000 inhabitants) was examined by 24-h monitoring of the blood pressure. The sample included an age-based cohort of patients from one general practitioner (men of 40-45, women of 45-50 years of age) without any previous diagnosis of essential hypertension.

The study was approved by the Committee for the Ethics of Medical Experimentation on Human Subjects, Medical Faculty, Masaryk University, Brno (No. 64/93, 1993). The undersigned informed consent of the examined subjects was obtained beforehand.

Analysis of 24-hour blood pressure monitoring

Each record consisted of 48-53 measurements of the systolic and diastolic pressure. The mean arterial pressure was calculated according to the algorithm of the measuring device producer's (Spacelab 90207). The daytime frequency of measurements was programmed on a three-measurements-per-hour basis from 06:00 to 22:00 h; the nighttime measurements were performed on a one-measurement-per-hour basis from 22:00 to 06:00 h. Records lacking three or more measurements during the daytime and two or more measurements during the nighttime were excluded.

Finally, 243 records of 124 men and 119 women were included in the statistical analyses. Individual 24-h BP measurements and/or approximating curves were characterized by group means of 14 parameters (Table 2). The following procedures for systolic, diastolic and mean arterial pressures were used. A set of measurements obtained for each individual was approximated for systolic, diastolic and mean arterial pressures (using the method of the least squares) by a combination of two sinusoids according to the equation

$$F(t) = BP + A_{BP}(24)\cos 2 \times (t - P(24))/24 + A_{BP}(12)\cos 4 \times (t - P(12))/24$$

where *BP* is the mean blood pressure (in mm Hg), $A_{BP(24)}$ and $A_{BP(12)}$ represent the values of its 24-h and 12-h amplitudes. Time *(t)*, and the time parameters (phase shifts) *P(24)* and *P(12)* are expressed in hours. The constant *24* represents 24 hours per day. The residual variances ("residua", Table 2) were calculated as the square root of the mean of the sum of squares of deviations of the individual readings from the two component sinusoid (24-h and 12-h) belonging to each individual. Differences in the phase shifts among the BP (systolic, mean arterial and diastolic) curves were not significant and, therefore, the shifts – separately for both rhythms *P (24)* and *P(12)* – were pooled together.

The first two harmonic sinusoid functions with periods of 24 and 12 hours represented a substantial part of the whole variance of the measured BP values (54 % and 58 % in the case of systolic and diastolic BP variance, respectively). The 12-h period was included as it itself accounted for 14 % and 15 % of the whole variance of systolic and diastolic BP, respectively.

Thus, a set of readings obtained from each individual was approximated for systolic, diastolic and mean arterial blood pressures (the method of least squares) by combining two sinusoid components with wavelengths of 24 hours and 12 hours. The 8-h and 6-h components were not considered because they were not significant.

Statistical analyses

In the following step, the blood pressure parameters were corrected for sex and body mass index using the multiple regression method. Then, the multiple analysis of variance for the corrected blood pressure parameters, I/D ACE gene polymorphism and ABO blood group system was performed to evaluate the main effects of I/D ACE gene polymorphism and ABO blood group system as well as the interaction of both factors.

Genetic analysis

The analysis I/D ACE gene polymorphism was performed according to Rigat *et al.* (1992). Oligonucleotides 5′ CTg gAg ACC ACT CCC ATC CTT TCT 3′ and 5′gAT gTg gCC ATC ACA TTC gTC AGA T 3′ were used as sense and anti-sense primers.

The PCR reaction was performed in a final volume of 50 μl containing 50 mmol KCl, 10 mmol TRIS-HCl buffer, pH 8.4, 5 mmol $MgCl_2$, 2 μg BSA, 10 pmol of primers, 0.5 mmol of each dNTP and 1.2 U of Taq polymerase (Fermentas Molecular Biology, Lithuania). The DNA fragments were amplified for 30 cycles with denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 2 min after initial denaturation at 97 °C for 7 min and with final elongation at 72 °C for 10 min. PCR products were analyzed using gel electrophoresis, i.e. a 190 bp fragment in the absence and a 490 bp fragment in the presence of insertion. Single bands were detected using ethidium bromide in UV light.

ABO typing was performed routinely in the Department of Blood Transfusion, Faculty Hospital, Brno-Bohunice.

Table 1. Demographic data

	BMI (kg/m^2)	Age (years)
Men (n = 124)	25.98 ± 3.03	43.16 ± 2.02
Women (n = 119)	24.97 ± 3.63	47.90 ±1.73
Total (n = 243)	25.49 ±3.37	45.48 ± 3.03

Data are means±S.D.

Table 2. Parameters describing 24-h blood pressure recordings

		Men	Women	Together	Together after correction for sex and BMI
n		124	119	243	243
Systolic BP (mm Hg)	SBP	121.85 ± 9.64	116.19 ± 8.62	119.08 ± 9.56	119.02 ± 9.04
Mean arterial pressure (MAP) (mm Hg)	MAP	91.20 ± 7.61	87.78 ± 7.32	89.52 ± 7.65	89.48 ± 7.40
Diastolic BP (mm Hg)	DBP	77.04 ± 7.23	73.14 ± 7.10	75.13 ± 7.41	75.09 ± 7.12
Amplitude of 24-h rhythm, systolic BP (mm Hg)	As24	10.99 ± 4.84	10.87 ± 5.24	10.93 ± 5.03	10.93 ± 5.01
Amplitude of 24-h rhythm, MAP (mm Hg)	Am24	10.17 ± 4.47	10.55 ± 4.67	10.35 ± 4.56	10.36 ± 4.53
Amplitude of 24-h rhythm, diastolic BP (mm Hg)	Ad24	10.18 ± 4.36	10.54 ± 4.28	10.36 ± 4.32	10.36 ± 4.28
Phase shift of 24-h rhythm (hr)	P24	13.92 ± 2.60	13.20 ± 2.17	13.57 ± 2.42	13.56 ± 2.39
Amplitude of 12-h rhythm, systolic BP (mm Hg)	As12	6.67 ± 3.06	5.56 ± 2.72	6.12 ± 2.94	6.11 ± 2.88
Amplitude of 12-h rhythm, MAP (mm Hg)	Am12	6.15 ± 3.11	5.40 ± 2.62	5.78 ± 2.90	5.78 ± 2.90
Amplitude of 12-h rhythm, diastolic BP (mm Hg)	Ad12	6.28 ± 3.15	5.26 ± 2.35	5.78 ± 2.83	5.77 ± 2.77
Phase shift of 12-h rhythm (hr)	P12	7.98 ± 1.60	7.37 ± 1.98	7.68 ± 1.82	7.68 ± 1.77
Residual variance, systolic BP (mm Hg)	Rs	12.33 ± 2.77	11.63 ± 3.04	11.99 ± 2.92	11.98 ± 2.87
Residual variance, mean arterial BP (mm Hg)	Rm	11.25 ± 2.58	10.88 ± 2.88	11.07 ± 2.73	11.06 ± 2.70
Residual variance, diastolic BP (mm Hg)	Rd	11.20 ± 2.51	10.65 ± 2.58	10.93 ± 2.55	10.92 ± 2.50

Data are means±S.D.

Results

The normotensive subjects (n = 243, Table 1) with complete records of 24-h BP monitoring were included in this study. The parameters of 24-h BP monitoring records were corrected for sex and BMI (Table 2) to exclude their possible influence on the relations to I/D ACE gene polymorphism and the ABO blood group system tested in the present study. The parameters of the 24-h blood pressure records corrected for sex and BMI were not associated with either I/D ACE gene polymorphism or the ABO blood group system separately (Table 3). However, a significant association was found between the interaction of I/D ACE gene polymorphism and ABO blood group system on the one hand and the mean values of systolic or mean arterial pressures and the phase shift of 24-h BP periods on the other hand (Table 4).

Table 3. Multivariational analysis of variance: main effects of ABO blood group system and I/D ACE gene polymorphism

Main effect: Dependent variable	ABO P =	I/D ACE P =
SBP	0.629	0.372
MAP	0.891	0.550
DBP	0.697	0.646
As24	0.942	0.924
Am24	0.809	0.526
Ad24	0.769	0.611
P24	0.470	0.093
As12	0.558	0.798
Am12	0.741	0.618
Ad12	0.626	0.795
P12	0.281	0.588
Rs	0.686	0.986
Rm	0.348	0.631
Rd	0.574	0.654

The mean values of BP parameters together with I/D ACE gene variants and the phenotypes of the ABO blood group system are presented in Table 5. Furthermore, the homozygous DD genotype is associated with the highest values of systolic blood pressure and mean arterial pressure in subjects with blood groups A and AB, while the DD genotype is accompanied by the lowest values of mean blood pressure among all I/D ACE genotypes in blood groups B and O.

The effect of the ABO blood group system on the phase shift of the 24-h BP rhythm is ambidirectional. A shift towards earlier time characterizing the DD genotype is expressed in the subjects with B, AB and O blood groups, while there is a shift towards a later time in the DD genotype of the A blood carriers. The possible maxima of the 24-h BP rhythm in the population are spanned between 12:37 (DD genotype in blood group O carriers) and 14:45 (ID genotype in blood group O carriers) (Table 5).

Table 4. Multivariational analysis of variance: interaction of the phenotype of ABO blood group system and I/D ACE gene polymorphism

Dependent variable	Mean Sqr Effect	Mean Sqr Error	F ratio (df = 1, 219)	P =
SBP	209.51	78.69	2.66	0.016
MAP	129.30	53.30	2.43	0.027
DBP	101.05	49.64	2.04	0.062
P24	12.55	5.50	2.28	0.037

Discussion

A chronobiological approach to the evaluation of BP records makes it possible to characterize the rhythmic (circadian and/or ultradian) oscillations as a substantial component of the whole daily variance of blood pressure. The standard approach, expressing the variance using means and standard deviations distorts the information.

An association of I/D polymorphism of the ACE gene with BP levels has been claimed by some (Zee *et al.* 1992, Morise *et al.* 1994), but has not been confirmed by other authors (Jeunemaitre *et al.* 1992, Higashimori *et al.* 1993, Harrap *et al.* 1993).

The significant association between the interaction of I/D ACE gene polymorphism and the ABO blood group system on the one hand and the mean values of systolic or mean arterial pressures and a phase shift of 24-h BP periods (corrected for sex and BMI) on the other hand, may be surprising. It represents an interesting parallelism to our previous finding (Cídl *et al.* 1994) of an independent effect of the ABO blood group system on ACE serum activity. The different glycosylation of the ACE molecule and/or of an other molecule of the RAS system (angiotensinogen) in different ABO blood groups seems to modulate the regulation of blood pressure in a given subject depending on the genetic variants of glycosylated genes.

Table 5. ABO blood group system, I/D ACE gene polymorphism and phase shifts of 24-h BP rhythm

Blood group	I/D ACE genotype	SBP (mm Hg)	MAP (mm Hg)	DBP (mm Hg)	Phase shift of 24-h BP rhythm (hours)
A	II	118.5	88.6	73.9	13: 43
A	ID	118.3	88.6	73.9	13: 26
A	DD	121.8	91.0	76.1	14: 07
B	II	118.6	91.5	77.6	13: 47
B	ID	121.7	91.3	77.0	12: 53
B	DD	115.7	87.6	74.0	12: 23
AB	II	112.2	83.5	70.5	13: 14
AB	ID	120.9	90.6	76.3	14: 33
AB	DD	124.7	94.5	80.1	13: 08
O	II	119.3	90.0	75.8	13: 57
O	ID	118.6	89.7	75.5	14: 45
O	DD	115.1	86.9	72.9	12: 37

The problem of genetic conditioning of the rhythmic aspects (*P24*) of blood pressure readings may even be important from the clinical point of view when the diagnosis of hypertension and/or timing of hypertensive therapy is to be decided.

The statistical significance of our results may naturally be discussed from the aspect of multiple comparison of the data. Though individual significances do not attain high values, it is important to consider the fact that as many as three significant probabilities of the fourteen calculated for blood pressure parameters were obtained

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