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ORIGINAL ARTICLE

Association of circadian genes with diurnal blood pressure changes and non-dipper essential hypertension: a genetic association with young-onset hypertension

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Recent studies have suggested that circadian genes have important roles in maintaining the circadian rhythm of the cardiovascular system. However, the associations between diurnal BP changes and circadian genes remain undetermined. We conducted a genetic association study of young-onset hypertension, in which 24-h ambulatory blood pressure (BP) monitoring was performed. A total of 23 tag single-nucleotide polymorphisms (SNPs) on 11 genes involved in circadian rhythms were genotyped for correlations with diurnal BP variation phenotypes. A permutation test was used to correct for multiple testing. Five tag SNPs within five loci, including rs3888170 in *NPAS2*, rs6431590 in *PER2*, rs1410225 in *RORββ*, rs3816358 in *BMAL1* and rs10519096 in *RORα*, were significantly associated with the non-dipper phenotype in 372 young hypertensive patients. A genetic risk score was generated by counting the risk alleles and effects for each individual. Genotyping was performed in an additional independent set of 619 young-onset hypertensive subjects. Altogether, non-dippers had a higher weighted genetic risk score than dippers (1.67 ± 0.56 *vs.* 1.54 ± 0.55, $P<0.001$), and the additive genetic risk score also indicated a graded association with decreased diurnal BP changes ($P=0.006$), as well as a non-dipper phenotype ($P=0.031$). After multivariable logistic analysis, only the circadian genetic risk score (odds ratio (OR), 1550; 95% confidence interval (CI), 1.225–1.961, $P<0.001$) and the use of β-blockers (OR, 1.519; 95% CI, 1.164–1.982, $P=0.003$) were independently associated with the presence of non-dippers among subjects with young-onset hypertension. Genetic variants in circadian genes were associated with the diurnal phenotype of hypertension, suggesting a genetic association with diurnal BP changes in essential hypertension.
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INTRODUCTION

Hypertension is a multifactorial genetic trait that can be influenced by differential gene–gene interaction, variable penetrance and various environmental factors. Although >20 genes have been assessed for their general genetic associations with essential hypertension,[1] the identification of an 'intermediate phenotype', such as a specific subgroup of hypertensive patients with more homogeneous baseline characteristics and genetic backgrounds, could have particular clinical significance. A cluster of candidate genes, which could be assessed and managed more reliably, might contribute to the determining potential pathophysiology and determining the clinical outcomes of these patients.

It is well known that blood pressure (BP) can vary diurnally and exhibit a decrease of >10%, a so-called 'dip', at nighttime compared

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with that in the daytime during our normal daily lives. With the use of an ambulatory BP monitor, we previously identified a subgroup of young hypertensive patients who did not experience normal nighttime decreases in BP, corresponding to so-called 'non-dipper' hypertension.[2] Compared with dipper hypertensive patients, non-dipper hypertensive patients had significantly more severe autonomic dysfunction, glucose metabolism abnormalities and vascular complications.[2] Accumulating clinical evidence has further indicated that blunted normal nocturnal BP variation could be associated with increased cardiovascular morbidity and mortality,[3] suggesting that non-dipper hypertensives might constitute a particular subgroup of hypertensive patients at high risk for future adverse events. Although early identification and risk stratification are mandatory, the underlying mechanisms linking hypertensive patients with the presence of non-dipper status are not adequately understood. Multiple possible pathophysiologies, such as abnormal neurohormonal regulation, lack of physical activity, increased dietary sodium intake, smoking and genetic predisposition, have been proposed.

Recently, the link between circadian clock function and metabolic diseases has attracted attention. Turek *et al.*[4] demonstrated that clock-mutant mice developed the features of metabolic syndrome, including hyperglycemia and hyperlipidemia. Ando *et al.*[5] demonstrated that rhythmic mRNA expression of clock genes was dampened in the peripheral leukocytes of patients with type 2 diabetes. In addition to metabolic syndrome and diabetes, genes governing circadian rhythms, such as *BMAL1*[6] and *NPAS2*,[7] have been reported to be associated with hypertension. However, whether circadian genes contribute to diurnal BP change remains to be determined. Heritability studies seeking genetic evidence of familial aggregation have suggested that diurnal variability in BP might be partially under genetic control.[8] Furthermore, melatonin, which modulates the central circadian rhythm, significantly affected the variability of BP.[9] Taken together, one could speculate about the potential role of genetic regulation of circadian rhythms in diurnal BP changes. To avoid the influence of the aging process and environmental factors, we focused particularly on young-onset hypertension, which is believed to involve a more prevalent genetic component in the pathogenesis of essential hypertension. We investigated whether circadian genes were associated with the presence of the non-dipper phenotype. Given the accumulating evidence linking non-dipper hypertension to elevated CVD risk, our findings in relatively young patients might provide specific genetic insights into early risk identification, stratification and management in clinical practice.[10]

METHODS

Patients

A total of 991 patients with young-onset hypertension were recruited for the Academia Sinica Multi-center Young-Onset Hypertension Genetic Study. The diagnostic criteria for young-onset hypertension have been previously reported.[11] Hypertensive patients were included if their systolic BP was >140 mm Hg and/or if their diastolic BP was >90 mm Hg over a period of 2 months or if they were taking any antihypertensive medications. All of the study patients had to have received their initial diagnoses of essential hypertension at between 20 and 51 years of age. Furthermore, upon enrollment, the potential causes of secondary hypertension, including chronic renal disease, renal arterial stenosis, primary aldosteronism, coarctation of the aorta, thyroid disorders, Cushing syndrome and pheochromocytoma, were carefully excluded by extensive review of medical records and by further clinical examinations and investigations, including blood chemistry and endocrinology tests for each patient. Patients diagnosed with diabetes mellitus (fasting glucose >126 mg dl^{-1}) or marked obese status (body mass index >35 kg m^{-2}) were also excluded from this study. The study protocol was approved by the Human Investigation Committee of the Institute of Biochemical Science, Academia Sinca, Taiwan, and all of the subjects provided written informed consent

Ambulatory BP monitoring

After enrollment, all of the patients underwent ambulatory BP monitoring for 24 h on a weekday with one of the three available automatic devices. The device was an Oscar oscillometric ambulatory BP monitor (Sun Tech Medical Instruments, Eynsham, Oxfordshire, UK). All of the patients were fitted with the device at between 0800 and 1000 h. The device was programmed to record BP and heart rate (HR) every 30 min throughout the day and night. BP and HR were measured automatically by detecting the Korotkoff sounds during stepwise deflations (3.0 ± 1.0 mm Hg per step). The accuracy of the BP monitoring devices was validated in our previous study.[12]

Diurnal BP change and the definition of non-dipper hypertension

For each recording, the study patients were advised to go to bed at 2300 h and to wake up at 0700 h. All medications were administered in the morning. The patients were also asked to record their real sleeping time if it was beyond this range. The BP measurements during sleep were then used as the nighttime BP, whereas the other BP recordings were used as the daytime BP. The difference between daytime BP and nighttime BP was calculated. In this study, the presence of non-dipper hypertension was defined a reduction in the mean BP of $<10\%$ at nighttime, compared with that in the daytime. Patients with non-dipper hypertension were identified as non-dippers.

Candidate gene selection and genotyping methods

Genomic DNA was extracted from peripheral blood using the Puregene DNA isolation kit (GentraSystems, Minneapolis, MN, USA). A two-stage genotyping approach was applied in this study. We used power calculation to calculate power of two-stage study.[13] When we assumed that an additive-effect disease model with a prevalence of 13.4% for young-onset hypertension,[14] a genetic relative risk of 2, and a disease allele frequency of 0.2–0.4, the power of our two-stage analysis was 0.87–0.90. Based on the results of power calculation, we divided all samples into two groups randomly to exam association between single-nucleotide polymorphisms (SNPs) of candidate genes and the diurnal BP pattern. During the first stage, a total of 23 tag SNPs on 11 genes involved in circadian rhythms were selected from the HapMap Project (International HapMap Consortium). These genes were *CLOCK*[15] (rs6811520, rs11932595, rs11931061), *BMAL1*[8] (rs11022742, rs7924734, rs3816358), *DEC1*[16] (rs1110261, rs908078), *DEC2*[16] (rs3809140), *CRY1*[17] (rs11113179, rs10861704), *PER1*[18] (rs2253820, rs2278637), *PER2*[19] (rs6431590, rs10462023), *NPAS1*[20] (rs1862499), *NPAS2*[7] (rs3888170, rs2305160), *RORα*[21] (rs17237318, rs10519096) and *RORβ*[22] (rs10512037, rs11144064). Genotyping assays were performed by the Academia Sinica National Genotyping Center (Taipei, Taiwan), using the Sequenom MassARRAY System (San Diego, CA, USA). Genotype calling was performed using the automatic call system implemented in the Sequenom Typer software (Sequenom). SNPs were excluded if the minor allele frequency was $<1\%$ or if it was not in Hardy–Weinberg equilibrium. During the second stage, SNPs that were significantly associated with the non-dipper phenotype were genotyped in another independent set of 619 patients with young-onset hypertension, to generate a genetic risk score for analyzing the genetic associations between circadian genes and the non-dipper phenotype.

Statistical analysis

The genotype frequency, allele frequency and Hardy–Weinberg *P*-value were calculated using Pearson's exact test. Allelic association analysis was performed using the X^2 test. The coefficients and odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated with logistic regression analysis. For the first stage of genotyping, risk factors, including sex, age, smoking habit and antihypertensive treatment, were controlled to determine the independent SNPs associated with non-dipper hypertension. Permutation tests based on 10 000 replications were also performed to correct the genotypic *P*-value using PLINK.[26] Analysis of a false discovery rate[23] using SAS GENETICS (SAS Institute Inc., Cary, NC, USA) was performed to account for a multiple test correction. The associations between non-dipper hypertension and SNPs were

evaluated by adjusting for multiple tests of all genes using a board threshold, false discovery rate *P*-value <0.1 in the first stage. The SNP for each individual gene that had a significant, independent association with the non-dipper phenotype was selected for second-stage genotyping. Joint analysis of the combined genetic information from both stages of genotyping (stage 1 and stage 2) was then performed to detect genetic associations, as previously prescribed.[13] A genetic score was generated by counting the risk alleles for each individual.[24] To assess further the impact of SNP markers in determining non-dipper status, weighted generic scores were generated, and non-dipper-associated SNP markers were entered into a multivariate regression model to obtain the regression coefficients for weighted genetic risk scores. The weighted sum of the risk allele account was calculated, and the weight for each SNP was the coefficient derived from the multivariate regression of these SNPs, as a previous study reported.[25] Logistic analyses were performed to investigate the factors that were independently associated with non-dipper hypertension after adjusting for risk factors, including age, sex, body mass index and antihypertensive agents. PLINK software[26] and SPSS software (v. 15.0, SPSS Inc, Chicago, IL, USA) were used for statistical analysis.

RESULTS

The basic characteristics of the 372 first-stage young-onset hypertension subjects are shown in Table 1. Young-onset hypertensive subjects with the non-dipper phenotype had higher baseline nighttime BP and smaller diurnal BP changes than those with dipper hypertension. Five tag SNPs within five loci, rs3888170 in *NPAS2* ($P=0.04$), rs6431590 in *PER2* ($P=0.02$), rs1410225 in *ROR*β ($P=0.039$), rs3816358 in *BMAL1* ($P=0.03$) and rs10519096 in *ROR*α ($P=0.01$), were significantly associated with the non-dipper phenotype in 372 young hypertensive patients (Table 2). These five SNPs were then genotyped in another independent set of 619 young-onset hypertension patients. There was no evidence of population stratification for these 1000 young-onset hypertensive subjects, who were assessed in our previous study.[27] Table 3 shows the effects of these five SNP associated with the non-dipper phenotype. Weighted generic scores were generated by counting the risk alleles and effects. A joint analysis of both stages of genotype information was performed to detect the genetic associations between circadian genes and the non-dipper phenotype.

Table 1 Characteristics of 372 patients in the first-stage genotyping study

	Dipper (N = *194*)	*Non-dipper* (n = *178*)	P-*value*
Age, years	37.36 ± 8.68	38.55 ± 8.65	0.187
BMI, kg m^{-2}	26.36 ± 3.84	25.97 ± 3.69	0.321
Systolic	125.34 ± 15.39	124.81 ± 14.42	0.737
Diastolic	83.22 ± 12.30	84.44 ± 11.48	0.325
Metabolic profiles			
Total cholesterol, mg dl^{-1}	197.04 ± 36.99	193.18 ± 36.56	0.312
HDL-C, mg dl^{-1}	46.28 ± 11.96	46.63 ± 12.07	0.777
Triglycerides, mg dl^{-1}	155.47 ± 110.31	144.76 ± 81.67	0.291
Glucose, mg dl^{-1}	96.72 ± 9.451	96.51 ± 8.53	0.827
Uric acid, mg dl^{-1}	6.74 ± 1.93	6.47 ± 1.71	0.156
Creatinine, mg dl^{-1}	0.83 ± 0.19	0.85 ± 0.18	0.537
Ambulatory BP monitor			
Awake			
Systolic BP, mm Hg	129.22 ± 13.19	124.67 ± 13.13	0.001
Diastolic BP, mm Hg	86.49 ± 10.38	84.07 ± 10.14	0.024
Sleep			
Systolic BP, mm Hg	110.50 ± 11.41	119.68 ± 13.55	<0.0001
Diastolic BP, mm Hg	71.15 ± 9.37	78.35 ± 10.70	<0.0001
Diurnal change, (%)	17 ± 5	4 ± 5	<0.0001
Antihypertensive agents			
ACEI+ARB, *n* (%)	61 (31.4)	60 (33.7)	0.641
β-Blockers, *n* (%)	68 (35.1)	80 (44.9)	0.052
Calcium channel blockers, *n* (%)	63 (32.5)	57 (32)	0.354
α-Blockers, *n* (%)	17 (8.9)	22 (12.3)	0.926
Diuretics, *n* (%)	9 (3.4)	5 (2.8)	0.258

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol.
Data are the mean ± s.d. and *n* (%).

Among the total 991 young-onset hypertensive patients, systolic BP and diastolic BP during the sleeping period were lower in patients with dipper hypertension than in those patients with non-dipper hypertension (109.77 ± 11.27 *vs.* 119.02 ± 12.77 mm Hg; 71.76 ± 9.08 *vs.* 78.05 ± 10.05 mm Hg, $P<0.001$, respectively; Table 4). In contrast, daytime systolic BP and diastolic BP, recorded by the ambulatory BP monitor, were higher in dippers than in non-dippers (128.29 ± 12.57 *vs.* 123.88 ± 12.82 mm Hg; 86.22 ± 9.73 *vs.* 83.6 ± 10.14 mm Hg, $P<0.001$, respectively). The diurnal BP change was greater in patients with dipper hypertension (17.06 ± 5.84% *vs.* 4.23 ± 4.47%, $P<0.001$) than in those with non-dipper hypertension. Moreover, there was more frequent use of β-blockade agents in patients with non-dipper hypertension than in those with dipper hypertension ($P<0.001$). Higher genetic risk scores were observed in patients with non-dipper hypertension than in those with dipper hypertension (1.67 ± 0.56 *vs.* 1.54 ± 0.55, $P<0.001$). Genetic risk scores were evenly distributed in the population (Figure 1a), and the cumulative incidence of patients with non-dipper phenotypes increased significantly with the score ($P=0.031$; Figure 1b). Furthermore, the percentage changes in diurnal BP decreased gradually as the genetic risk score increased ($P=0.006$; Figure 1c), indicating that the genetic risk score was associated with dampened normal diurnal blood changes, as well as the with non-dipper phenotype. When compared with a genetic score of 1.6–2.0, which was the median score, the risk difference was nine times higher in the highest genetic score group than in the lowest genetic score group, and the adjusted ORs for the presence of non-dipper hypertension increased significantly with the genetic risk score (Figure 2). After multivariate analysis adjusted for risk factors and antihypertensive agents, only the genetic risk score (OR, 1550; 95% CI, 1.225–1.961, $P<0.001$) and the use of β-blockers (OR, 1.519; 95% CI, 1.164–1.982, $P=0.003$) were independently associated with the presence of non-dipper status among subjects with young-onset hypertension (Table 5).

DISCUSSION

This study is the first study especially using candidate gene approach focusing on young-onset hypertensive subjects and ambulatory BP monitor to evaluate the association between circadian genes and diurnal BP change. In this study, we generated a genetic score to test the predicted value of genetic score in determining non-dipper hypertension. The additive circadian genetic score was significantly associated with the diurnal change in BP as well as the presence of non-dipper hypertension. Interestingly, both the use of β-blockers and the genetic risk score were independently associated with the presence of the non-dipper phenotype, suggesting complex interactions among genetics, drugs and diurnal BP changes in essential hypertension.

Circadian clock system for cardiovascular physiology

Cardiovascular or hemodynamic parameters, such as HR and BP, exhibit variations that are consistent with circadian rhythms.[28]

Table 2 The association between circadian genes and non-dipper hypertension

					Genotype frequency								
					No risk allele		*1-Risk allele*		*2-Risk alleles*				
Chr	*Locus*	*SNP*	*Locus relative to gene*	*Risk allele (genotype)*	*Dipper*	*Non-dipper*	*Dipper*	*Non-dipper*	*Dipper*	*Non-dipper*	*OR*	P-*value*	*FDR*
4	*CLOCK*	rs6811520	Intron	T (C/T)	82 (42.3)	77 (43.3)	90 (46.4)	75 (42.1)	22 (11.3)	26 (14.6)	1.05 (0.72–1.42)	0.75	0.92
4	*CLOCK*	rs11932595	Intron	A (G/A)	4 (2.1)	0 (0)	29 (14.9)	30 (16.9)	161 (83.0)	148 (83.1)	1.15 (0.69–1.90)	0.60	0.66
4	*CLOCK*	rs11931061	Intron	G (G/A)	82 (42.3)	77 (43.3)	90 (46.4)	75 (42.1)	22 (11.3)	26 (14.6)	1.05 (0.72–1.42)	0.74	0.92
11	*BMAL1*	rs11022742	Promoter	C (C/T)	58 (29.9)	52 (29.2)	91 (46.9)	83 (46.6)	45 (23.2)	43 (24.2)	1.03 (0.77–1.38)	0.82	0.94
11	*BMAL1*	rs7924734	Intron	G (G/A)	62 (32.0)	58 (32.6)	89 (45.9)	75 (42.1)	43 (22.2)	45 (25.3)	1.05 (0.78–1.40)	0.73	0.92
11	*BMAL1*	rs3816358	Intron	T (T/G)	137 (70.6)	112 (62.9)	53 (27.3)	54 (30.3)	4 (2.1)	12 (6.7)	1.50 (1.04–2.18)	0.03	0.08
17	*PER1*	rs2253820	Coding exon	A (A/G)	24 (12.4)	25 (14.0)	82 (42.3)	71 (39.9)	88 (45.4)	82 (46.1)	1.02 (0.75–1.39)	0.89	0.97
17	*PER1*	rs2278637	Promoter	T (T/G)	48 (24.7)	44 (24.7)	103 (53.1)	85 (47.8)	43 (22.2)	49 (27.5)	1.11 (0.84–1.49)	0.46	0.56
2	*PER2*	rs6431590	Intron	A (A/G))	96 (53.9)	82 (42.3)	70 (39.1)	91 (46.9)	12 (6.7)	21 (10.8)	1.45 (1.06–1.99)	0.02	0.05
2	*PER2*	rs10462023	Intron	A (G/A)	10 (5.6)	8 (4.1)	69 (38.8)	76 (39.2)	99 (55.6)	110 (56.7)	1.07 (0.77–1.50)	0.68	0.92
3	*DEC1*	rs1110261	Promoter	C (C/T)	119 (66.9)	130 (67)	54 (30.3)	54 (27.8)	3 (1.7)	9 (4.6)	1.12 (0.88–1.63)	0.57	0.92
3	*DEC1*	rs908078	Coding exon	T (T/C)	10 (5.2)	5 (2.8)	66 (30.4)	58 (32.6)	118 (60.8)	115 (64.6)	1.21 (0.84–1.71)	0.30	0.41
12	*DEC2*	rs3809140	Promoter	T (T/C)	149 (76.8)	127 (71.3)	43 (22.2)	48 (27.0)	2 (1.0)	3 (1.7)	1.30 (0.85–1.98)	0.22	0.384
19	*NPAS1*	rs1862499	Downstream	G (G/A)	22 (11.3)	16 (9.0)	78 (40.2)	66 (37.1)	94 (48.5)	96 (53.9)	1.21 (0.88–1.66)	0.24	0.78
2	*NPAS2*	rs3888170	Intron	C (C/T)	63 (35.4)	54 (27.8)	94 (52.8)	103 (53.1)	21 (11.8)	54 (19.1)	1.36 (1.01–1.82)	0.04	0.05
2	*NPAS2*	rs2305160	Coding exon	C (C/T)	133 (74.7)	139 (71.6)	42 (23.6)	52 (26.8)	3 (1.7)	3 (1.5)	1.13 (0.75–1.70)	0.57	0.92
12	*CRY1*	rs11113179	Intron	T (T/C)	138 (71.1)	124 (69.7)	50 (25.8)	51 (28.7)	6 (3.1)	3 (1.7)	1.00 (0.68–1.48)	0.99	0.99
12	*CRY1*	rs10861704	Promoter	A (A/G)	116 (59.8)	98 (55.1)	66 (34.0)	74 (41.6)	12 (6.2)	6 (3.4)	1.06 (0.75–1.48)	0.76	0.76
15	*RORA*	rs17237318	Intron	T (T/C)	17 (8.7)	7 (3.9)	69 (35.6)	60 (33.7)	108 (55.7)	111 (62.4)	1.35 (0.96–1.90)	0.08	0.31
15	*RORA*	rs10519096	Intron	A (A/G)	155 (79.9)	123 (69.1)	39 (20.1)	52 (29.2)	0 (0)	3 (1.7)	1.74 (1.13–2.69)	0.01	0.07
9	*RORB*	rs10512037	Intron	G (G/A)	58 (29.9)	55 (30.9)	97 (50.0)	85 (47.8)	39 (20.1)	38 (21.3)	1.01 (0.75–1.34)	0.97	0.99
9	*RORB*	rs11144064	3′-UTR	T (T/C)	85 (43.8)	74 (41.6)	90 (46.4)	85 (47.8)	19 (9.8)	19 (10.7)	1.07 (0.79–1.45)	0.65	0.92
9	*RORB*	rs1410225	Downstream	C (C/T)	52 (26.8)	25 (14.0)	82 (42.3)	94 (52.8)	60 (30.9)	59 (33.1)	1.36 (1.01–1.81)	0.039	0.08

Abbreviations: FDR, false discovery rate; OR, odds ratio; SNP, single-nucleotide polymorphism; UTR, untranslated region.

Table 3 The effect of genetic association of circadian genes and non-dipper status in young-onset hypertension

SNP	*Chromosome*	*Gene*	*Effect allele*	*β (s.e.)*	P-*value*
rs3816358	*11*	*BMAL1*	T	0.436 (0.193)	0.024
rs6431590	2	*PER2*	A	0.364 (0.170)	0.032
rs3888170	2	*NPAS2*	C	0.365 (0.163)	0.025
rs10519096	15	*RORA*	A	0.621 (0.241)	0.010
rs1410225	9	*RORB*	C	0.318 (0.152)	0.037

Abbreviation: SNP, single-nucleotide polymorphism.

Table 4 Characteristics of the 991 total patients with young-onset hypertension (joint analysis of both stages of genotyping experiments)

	*Dipper (*N = *516)*	*Non-dipper (n = 475)*	P-*value*
Male, *n* (%)	370 (71.7)	310 (65.3)	0.029
Age, years	40.33 ± 7.38	41.46 ± 7.09	0.015
BMI, kg m^{-2}	26.42 ± 3.48	26.49 ± 3.48	0.770
Waist circumference, cm	88.76 ± 9.48	88.44 ± 10.03	0.612
BP, mm Hg			
Systolic	126.63 ± 15.08	125.51 ± 14.42	0.234
Diastolic	85.16 ± 11.81	84.46 ± 11.7	0.348
Metabolic profiles			
Total cholesterol, mg dl^{-1}	195.49 ± 36.23	195.23 ± 35.91	0.908
HDL-C, mg dl^{-1}	46.19 ± 12.49	45.21 ± 11.87	0.209
Triglycerides, mg dl^{-1}	161.03 ± 109.51	165.99 ± 109.4	0.476
Glucose, mg dl^{-1}	98.19 ± 9.73	97.31 ± 8.82	0.140
Uric acid, mg dl^{-1}	6.66 ± 1.77	6.6 ± 1.7	0.580
Creatinine, mg dl^{-1}	0.84 ± 0.18	0.85 ± 0.20	0.422
Ambulatory BP monitor			
Awake			
Systolic BP, mm Hg	128.29 ± 12.57	123.88 ± 12.82	<0.0001
Diastolic BP, mm Hg	86.22 ± 9.73	83.64 ± 10.14	<0.0001
Sleep			
Systolic BP, mm Hg	109.77 ± 11.27	119.02 ± 12.77	<0.0001
Diastolic BP, mm Hg	71.76 ± 9.08	78.05 ± 10.05	<0.0001
Diurnal change (%)	17.06 ± 5.84	4.23 ± 4.47	<0.0001
Antihypertensive agents			
ACEI+ARB, *n* (%)	181 (35.1)	176 (37.1)	0.518
β-Blockers, *n* (%)	205 (39.7)	240 (50.5)	0.001
Calcium channel blockade, *n* (%)	196 (38)	179 (37.7)	0.922
α-Blockers, *n* (%)	54 (10.5)	51 (10.7)	0.890
Diuretics, *n* (%)	20 (3.9)	15 (3.2)	0.541
Weighted genetic score	1.54 ± 0.55	1.67 ± 0.56	<0.0001

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol.
Data are the mean ± s.d. and *n* (%).

Cardiovascular events, including myocardial infarction, stroke and sudden cardiac death, demonstrate marked circadian variations,[29] and patients with impaired circadian patterns in BP are at increased risk for cardiovascular complications.[3] The circadian system is responsible for regulating physiological rhythms, and it consists of multiple negative and positive regulatory pathways.[30] The circadian system includes the transcriptional–translational autoregulatory loop, which is located in the suprachiasmatic nucleus within the hypothalamus in the brain, which generates the molecular oscillations of circadian genes that regulate downstream oscillators in peripheral tissues.[30] In brief, *CLOCK* and *BMAL* induce the expression of several genes by binding to E-box enhancer elements in the promoters of target genes, including *Period* genes (*PER1/2/3*) and *Cryptochrome* genes (*CRY1/2*), the protein products of which are PER and CRY, respectively, which negatively regulate the activation of their own expression by inhibiting the activity of the *CLOCK/BMAL1* complex, thereby constituting a negative feedback loop.[31] Another regulatory loop is induced by *CLOCK/BMAL1* heterodimers that activate the transcription of the retinoic acid-related orphan nuclear receptors *REV-ERB*αα and *ROR*α.[32,33] Members of *ROR* (α, β *and* γ) and *REV-ERB* (α *and* β) could regulate *BMAL1*.

Circadian genes and non-dipper hypertension

This study first showed the associations between circadian genes and non-dipper hypertension. Candidate genes, including *CLOCK*, *BMAL1, PER1/2, CRY1/2, DEC1/2, NPAS1/2* and *ROR* (α *and* β), which were either directly or indirectly involved in circadian rhythms, were selected for our study. SNPs in *BMAL1* were recently identified as susceptible genetic markers for hypertension in a family association study.[6] *NPAS2* was also reported to be associated with hypertension.[7] Animal studies have shown that altered *Bmal1* levels could contribute to hypertension in spontaneously hypertensive rats by producing aberrant sleep patterns and changes in the macrostructure of sleep.[34] It is believed that sleep disruptions have an important role in hypertensive models.[35] Modification of the *NPAS2* gene could alter sleep patterns, and the physiologic function of *PER2* influences behavior sensitization to psychostimulants.[9] Animal studies have demonstrated that ablating core clock genes causes either an attenuated or a flattened BP rhythm in mice,[36] suggesting the importance of the circadian clock in BP regulation.

Our study showed that risk alleles in the *NPAS2, PER2, ROR*α, *ROR*β and *BMAL1* genes were associated with an increased risk of non-dipper hypertension. Patients with more risk alleles were at greater risk than those with fewer risk alleles. However, the mechanisms underlying 'circadian genes' and their involvement in regulating BP, leading to cardiovascular complications, remain undetermined.[37] Barclay *et al.*[38] recently demonstrated that circadian desynchrony caused by shiftwork in a mouse model could cause disruption of diurnal liver transcriptome rhythms, enriching the pathways involved in glucose and lipid metabolism. This study result provided a possible linking between the circadian clock and cardiovascular risk. In addition, Burgueño *et al.*[39] showed increased serum levels of resistin, a cardiovascular biomarker, in rotating shiftworkers. Furthermore, Sookoian *et al.*[40] reported that rotating shiftworkers carried a higher risk of metabolic syndrome and higher systemic inflammation, independent of age or physical activity. Together, the circadian clock contributes to cardiovascular events that could involve pathways of metabolism and systemic inflammation. Recent studies have demonstrated that peroxisome proliferator-activated receptors (PPARs) interact with the molecular clock components and have important roles in maintaining cellular energy metabolism.[41] PPARα could induce BMAL1 gene expression,[42] and the transcription of PPARα was also regulated by the CLOCK-BMAL1 complex.[43] In addition, PPARγ, which controls lipid metabolism, has been reported to regulate the transcription of the BMAL1 and Rev-erbα genes.[44,45] In addition, it has also been reported that *PER2* participates in cellular energy homeostasis and that it is regulated by *PPAR*α[46] and *PPAR*γ.[47] Interestingly, a recent animal study indicated that increased PPARγ

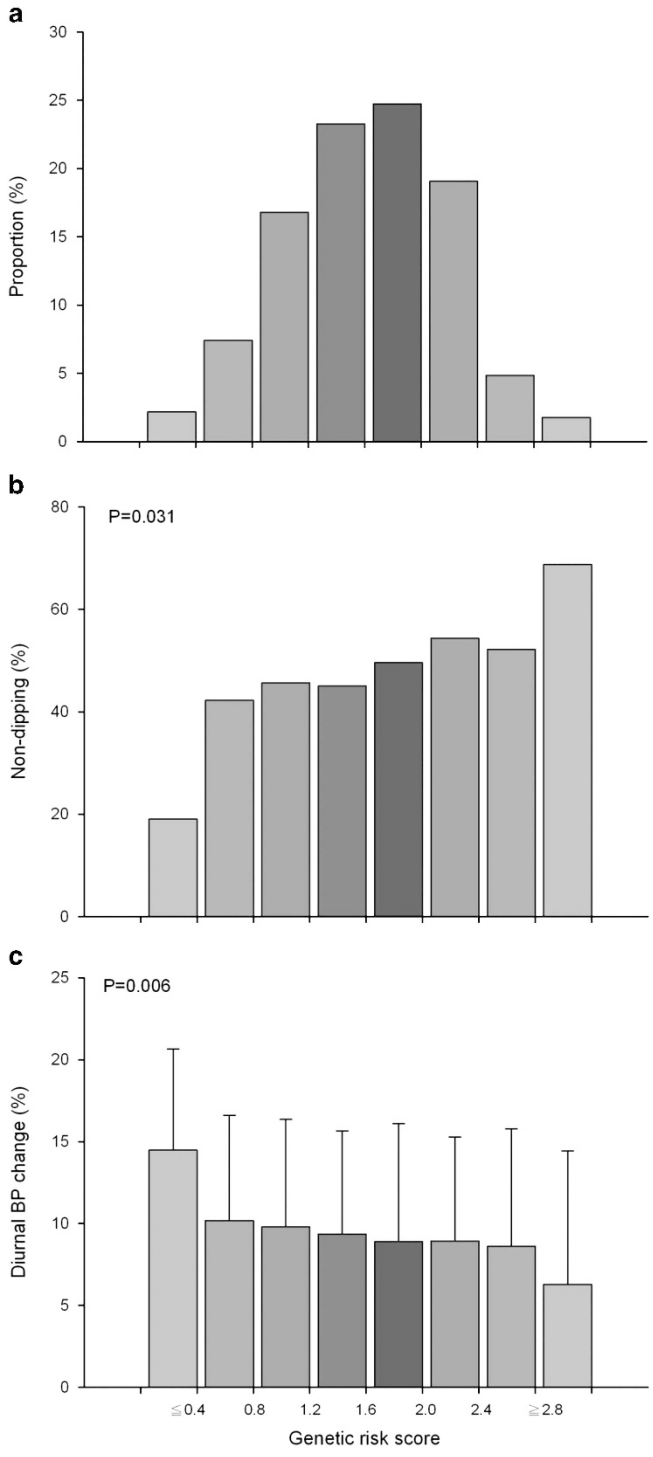

Figure 1 Additive gene risk scores in patients with non-dipper hypertension: (**a**) proportion of the genetic risk score; (**b**) prevalence of non-dipper hypertension; (**c**) diurnal blood pressure (BP) change (%).

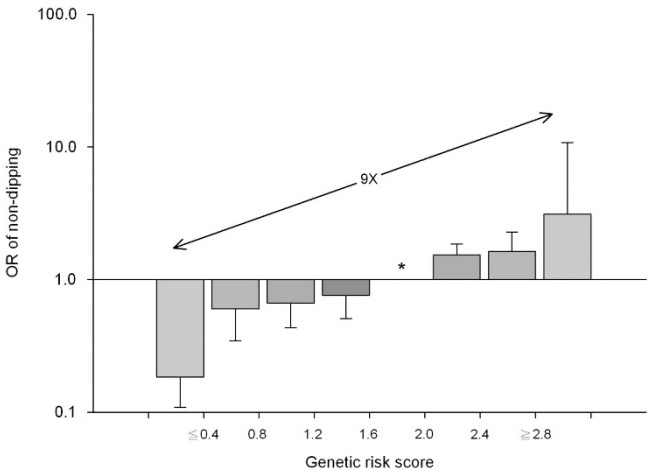

Figure 2 Odds ratio (OR) of the non-dipper phenotype adjusted for age, sex, body mass index and antihypertensive medications. * indicates referent.

Table 5 Independent predictors for non-dipper hypertension

Parameters	*Odds ratio (95% CI)*	P-*value*
Male gender	0.755 (0.562–1.013)	0.061
Age, years	1.00 (0.997–1.035)	0.092
Total cholesterol, mg dl^{-1}	1.00 (0.996–1.003)	0.911
Triglyceride, mg dl^{-1}	1.00 (0.999–1.002)	0.725
SBP, mm Hg	0.993 (0.980–1.007)	0.331
DBP, mm Hg	1.007 (0.990–1.024)	0.424
BMI, kg m^{-2}	1.013 (0.976–1.053)	0.493
Circadian genetic risk score	1.550 (1.255–1.961)	<0.001
ACEI /ARB	1.148 (0.873–1.511)	0.323
β-Blocker	1.519 (1.164–1.982)	0.002
Calcium channel blockade	0.931 (0.708–1.224)	0.609
α-Blocker	0.996 (0.653–1.520)	0.987
Diuretics (thiazide)	0.828 (0.406–1.685)	0.602

Abbreviations: ACEI, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

expression contributed to decreased BP in mice.[48] Pioglitazone, a ligand of PPARγ used to control blood sugar, has been reported to decrease BP independent of reductions in blood sugar levels, and it could even restore circadian rhythms.[49] Furthermore, it has been reported that the PPAR gene was associated with hypertension.[50,51] Altogether, circadian genes might interact with PPARs and have important roles in maintaining cellular energy homeostasis and circadian rhythms. Further studies are required to investigate the roles of these genes in BP regulation.

β-Blockers and non-dipper hypertension

In this study, in addition to the genetic risk score, the use of β-blockers was found to be independently associated with the presence of non-dipper hypertension, which is consistent with the findings of Stanton's study. In that study, the author demonstrated a tendency for β-blockers to reduce daytime BP but not affect nighttime BP or early morning BP surges.[52] β-Blockers might also decrease the HR to a greater extent during the daytime. Some agents with partial agonist activity, such as pindolol, have even been reported to increase the HR at night.[53] Thus, the current evidence indicates that the BP-lowering effects of β-blockers are dominant during the daytime, but they are minor during the nighttime and early morning.[54] This evidence is consistent with our findings that the use of β-blockers was independently associated with a decrease in diurnal BP variability and that it could contribute to the development of non-dipper hypertension.

Furthermore, a β2-adrenoceptor agonist was reported to induce *PER1* expression,[55] indicating that β2-adrenoceptor modulation might alter the functions of circadian genes. Further studies are required to investigate these complex interactions.

Study limitations

There were some limitations of our study. First, as this study was conducted in Chinese hypertensive subjects, concerns might remain regarding the application of the results of our study to the general population without further replication studies. Second, the effects of lifestyle behaviors on BP control were considered, but it was difficult to collect the necessary information for our study. Subjects with relatively less healthy lifestyle behaviors might be more susceptible to genetic risk than those with more healthy behaviors.[56] Whether the genotype score has value in predicting the risk of non-dipper hypertension in specific subgroups of risk remains to be tested. Third, we used additional 619 patients to verify the association of the five SNPs with the diurnal BP pattern, but *P*-values were not enough significant after multiple test correction. Then, we used joint analysis instead of replication-based analysis to confirm the first-stage results. It has been mentioned that joint analysis is more efficient and more powerful than replication-based analysis for two-stage genome-wide association studies and similar joint analysis has been used in many genetic studies.[13] Furthermore, a small number of patients took thiazide diuretics and/or β-blockers, which might potentially have influenced the subsequent analysis of the interactions between antihypertensive medications and the circadian genetic risk score. In addition, the selected SNPs in this study might have been insufficient to encompass all of the genetic information of the circadian genes. Further fine-mapping and functional studies are required to identify the most critical regions.

CONCLUSION

Genes involved in circadian rhythms could be related to diurnal BP changes, as well as to the presence of non-dipper hypertension. The circadian genetic risk score and the use of β-blockers were found to be associated independently with non-dipper hypertension after adjusting for cardiovascular risk factors. These findings could be helpful in identifying at-risk subgroups among hypertensive patients and in providing additional information for the use of antihypertensive medications and the application of treatment strategies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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