GW26-e1335
siRNA Inhibits AT2 Receptor in Decreasing NO Generation by Recombinant Human Angiotensin Converting Enzyme 2 in Cardiac Microvascular Endothelial Cells
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OBJECTIVES SI RNA was used to silence AT2 receptor to explore the effect of Ang (1-9) -ACE2-AT2 pathway on NO formation after the impact of recombinant human Angiotensin Converting Enzyme 2 (rhACE2) on the cardiac microvascular endothelial cells (CMVEC).

METHODS Human cardiac microvascular endothelial cells (CMVEC) were cultured in vitro and grouped as follows: (1) The control group: normal CMVEC; (2) AngII intervention group: on the basis of the control group, AngII (1×10-6 mol/L) was added and incubated 24h; (3) On the basis of AngII intervention, rhACE2 was added for incubation 5, 10, 15, 30, and 60 min respectively; (4) AT2 receptor inhibitor group: based on AngII intervention, AT2 receptor inhibitor (10μmol/L) was added for incubation 30min, and then rhACE2 (100 μmol/L) was added for incubation 30min, then rhACE2 (100 μmol/L) was added for incubation 30min. Also a negative siRNA control group (negative control, NcsiRNA) was set up: after NcsiRNA transfection, it was treated as described above. Griess reagent measurement was applied to detect NO content in cell culture supernatant, RT-PCR to detect the expression of eNOS mRNA in HUVEC, Western blot to detect the expression of phosho-eNOS. NO fluorescent probe DAF-FM DA was loaded to detect intracellular NO formation and the activity of endothelial nitric oxide synthase (eNOS).

RESULTS The content of NO in AngII intervention group (3.495 ± 0.362 nmol/L) was significantly lower than that in the control group (11.513 ± 0.392) (P <0.05). After rhACE2 treatment, the NO contents and the phosphor-eNOS expression levels of cultured cell liquid in subgroups were significantly higher than those in AngII intervention group (P <0.05). However the protein expression levels of eNOSmRNA and non-phosho-eNOS showed no significant difference compared with AngII intervention group (P > 0.05). And after CMVEC was intervened by AT2 pathway inhibitor (PD123319), the expression levels of phosho-eNOS were significantly lower than those in rhACE2 30min treated group (P <0.05). After the successful transference of siRNA into CMVEC, Western blot test results showed that 48 h after transfection, the protein expression of AT2 receptor decreased (P <0.05). Compared with non-transfected control group and negative control group, the eNOS activity and NO levels of the AT2 siRNA transfected group were significantly reduced.

CONCLUSIONS Ang (1-9) -ACE2-AT2 signaling pathway is important in rhACE2’s promotion of the activity of human cardiac microvascular endothelial cell eNOS and the NO formation.

GW26-e0487
Relationship between Blood Pressure Circadian Rhythm and Early Renal damage in the patients with Primary Hypertension
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OBJECTIVES To investigate the relationship between blood pressure circadian rhythm and early renal injury for the patients with primary hypertension.

METHODS A total of 225 hypertensive patients were divided into two groups according to nocturnal blood pressure decline rate (<10% into non-dippers and ≥10% into dippers). The nocturnal blood pressure decline rate, 24 h blood pressure (24h-PP) and blood pressure index (PPI) were determined according to the data from ambulatory blood pressure monitoring. The glomerular filtration rate (eGFR) was calculated by the MDRD and Cockcroft-Gault equations respectively. Fasting plasma glucose, BUN, Scr, Cys-C, TG, TC, LDL-C, HDL-C, UA and MAU were dynamically monitored and body mass index (BMI) was measured. The relationship between blood pressure circadian rhythm and early renal damage in the patients with primary hypertension was analyzed by using the univariate and multivariate regression methods. For all tests, P <0.05 was considered to be statistically significant.

RESULTS The non-dipper group (n=149) has significantly lower eGFR level (80.6±21.8 vs. 97.3±24.2 mL/min by MDRD equation, P<0.001; 70.4±19.2 vs. 81.2 mL/min by Cockcroft-Gault equation, P<0.001), but significantly higher MAU (15.6±9.9 vs. 12.0±7.9 mg/L, P<0.012) and PPI (0.42±0.07 vs. 0.39±0.06, P<0.001) were inclined to arteriosclerosis. The multivariate correlation and logistic regression analyses demonstrated that the N-SBP was correlated to MAU; BUN, Cys-C and PPI were correlated to eGFR based on the calculation with MDRD equation; and the Cys-C, D-DBP, 24-DBP, UA and BUN were correlated to eGFR based on the calculation with Cockcroft-Gault equation.

CONCLUSIONS The behavior of the early renal injury was significantly different between the non-dipper and dipper groups, which indicates the abnormal circadian rhythm of blood pressure could increase the renal target organ damage.

GW26-e1287
Aortic stiffness is associated with the central retinal arteriolar equivalent and retinal vascular fractal dimension in a population along the southeastern coast of China
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OBJECTIVES The objective of this study was to evaluate the association of the central retinal arteriolar equivalent (CRAE) and the retinal 2. We compared the differences of office blood pressure, 24 hour ambulatory blood pressure and left ventricular hypertrophy, vascular stiffness and urine protein among groups of different sodium intake.

RESULTS 24 hour sodium excretion formulas was obtained using SMU and PMU respectively, which have good consistency. The difference between the estimated and measured values in sodium excretion is 12.66 mmol/day (SMU) and 9.41 mmol/day (PMU), to be equal to 0.7 (SMU) and 0.6 g (PMU) salt intake. Comparing with Kawasaki and Tanaka method, the new formula shows the lower degree of deviation, and higher accuracy and precision. Blood pressure of high urinary sodium group is higher than that in low urinary sodium group (P<0.05). Left ventricular hypertrophy and urinary albumin / creatinine aggravated with the salt intake increase, this has eliminated the influence of other factors. All of morphologies of the relationship between ambulatory arterial stiffness index, pulse wave velocity and carotid intima-media thickness with quartiles of sodium intake resembled a J-shaped curve.
vascular fractal dimension, two quantitative parameters that reflect microcirculation, were aortic stiffness.

METHODS
In this cross-sectional study, we identified the cardiovascular risk factors in 2169 subjects using a health questionnaire, physical examinations and laboratory examinations. We evaluated the aortic stiffness using noninvasive brachial-ankle pulse wave velocity (baPWV) and assessed the microcirculatory alterations with CRAE and retinal vascular fractal dimension, which were measured using fundus photography and semiautomatic quantitative software, respectively.

RESULTS
The increase in baPWV (Q1-Q4) correlated with an increased likelihood of the central retinal artery narrowing and a reduction in the retinal vascular fractal dimension. Further adjustment of the cardiovascular risk factors diminished the beta-2-adrenergic effects on baPWV and CRAE, but increased the association between baPWV and retinal vascular fractal dimension.

CONCLUSIONS
Elevated baPWV correlates with reduced CRAE and retinal vascular fractal dimension. Such a finding supports microcirculation- and microcirculation-associated hypotheses.

GW26-e1391
Activated Effect of β-Estron on BKCa in Mesenteric Artery Smooth Muscle Cells of Pre-menopause and Post-menopause Women
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OBJECTIVES
Epidemiologic studies indicate that gender differences exist in essential hypertension. Premenopausal women have a much reduced incidence of hypertension compared with age-matched men. However, post-menopausal women develop increased incidence from hypertension. Laboratory research suggests that estrogen has beneficial cardiovascular effects through their ability to modulate their function; however, these mechanisms remain incompletely understood. So we isolated smooth muscle cells on women mesentery artery using acute enzyme method, recorded large-conductance Ca2+-activated potassium channel currents using perforate whole cell patch technique and observed the effect of β-estrone on BKCa in mesenteric artery smooth muscle cells of pre-menopause and post-menopause women.

METHODS
To apply acute enzyme method to isolate women mesenteric artery smooth muscle cells and record large-conductance Ca2+-activated potassium channel currents using perforate whole cell patch technique, and to examine the effects of β-E on BKCa of women mesenteric artery vascular smooth muscle cells (VSMCs) of pre-menopause non-hypertension group (PNH), post-menopause non-hypertension group (NH) and post-menopause women essential hypertension group (EH) to explore the relation among β-E, BKCa and women essential hypertension, and to identify that the mechanisms of effect of β-E on pre-menopause and post-menopause women essential hypertension.

RESULTS
(1) Comparisons of effects of estrone on BKCa macroscopic currents between PNH, NH and EH groups: (1) At -60 mV, the current densities of BKCa of PNH group increased 0.97±0.40 times after adding 100 μM β-E, β-E at -60 mV, the current densities of BKCa of NH group increased 0.75±0.47 times after adding 100 μM β-E, β-E at -60 mV, the current densities of BKCa of EH group increased 0.60±0.33 times after adding 100 μM β-E. (2) Effects of ICI 182780 on BKCa of women mesenteric artery smooth muscle cells. At -60 mV, the current densities of BKCa of women mesenteric artery smooth muscle cells could increase from 15.89±6.47 pA/pF to 27.88±6.75 pA/pF (P<0.001, n=23). (3) There was no inhibitory effect on BKCa after adding ICI 182780 subsequently. The current densities of BKCa of women mesenteric artery smooth muscle cells could decrease to 20.55±5.1 pA/pF (P<0.05, n=23).

CONCLUSIONS
(1) β-E could activate BKCa macroscopic currents on PNH group, NH group, EH group. Compared with PNH group, the effect of estrone on BKCa in NH was lower. That suggest β-E play an important role in pre-menopause women’ heart protection. Compared with NH group, the effect of estrone on BKCa in EH was lower, these data suggest that the responsiveness of effect of estrone was lower on blood vessel after hypertension and menopause was a factor of happening of hypertension; (2) β-E could make women mesenteric artery relax, and the effect could partly be inhibited by ICI 182780, so BKCa and ER were involved mainly in the mechanisms of E-induced relaxation in mesenteric artery.

GW26-e1402
Association of G-protein beta3 subunit gene CR25T polymorphism with cardiovascular events in Chinese hypertensive patients
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OBJECTIVES
The β3 subunit of heterotrimeric G protein-(-protein) encoded by gene GNB3 is crucial for cell signal transducers and a CR25T polymorphism in exon 10 of this gene is associated with increased intracellular signal transduction. Several recent studies conducted in normal population showed that GNB3 gene CR25T polymorphism is related to cardiovascular diseases (CVD). However, it is unclear whether β25T allele influences the incidence of CVD in patients with hypertension.

METHODS
In current study, 695 patients with essential hypertension were genotyped for CR25T polymorphism of GNB3 gene and followed up for 8 years to detect major adverse cardiac and cerebrovascular events (MACCEs) which include new onset of stroke, the onset of CVD and death. Established cardiovascular risk factors were used to adjust the multivariate Cox analysis for confounders.

RESULTS
After a mean follow-up period of 7.60±1.12 years, CVD was observed in 15 patients of the TT genotype group, 33 of the CC genotype group and 21 of the CT genotype group (37.9% vs. 9.9% vs. 7.5%; P=0.021). The time-to-event analysis using the Kaplan–Meier method showed a significantly higher incidence of MACCEs in the TT genotype group than those of the other genotypes (log rank P=0.03 and P=0.0001 for among the three genotypes and between the CC+ CT vs. TT genotypes respectively). In Cox analysis, the GNB3 gene 825TT variant was significantly and independently predictive of MACCEs (relative risk=2.579; P<0.0001), CVD (relative risk=2.982; P=0.0001), but not stroke (P=0.378), CVD, stroke (P=0.378) and death (P=0.827) after adjustment for age, Body Mass Index (BMI), presence or absence of diabetes mellitus, presence or absence of dyslipidemia and current smoking.

CONCLUSIONS
The GNB3 825 TT genotype may be a risk factor for CVD independent of other established cardiovascular risk factors in patients with essential hypertension. Further studies are needed to clarify the nature and pathways of this association.

GW26-e2433
Prevalence of Liddle syndrome among young hypertension patients of undetermined cause in a Chinese population
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OBJECTIVES
Liddle syndrome, an autosomal dominant form of monogenic hypertension, has been regarded as a rare disorder, which leads to many Liddle syndrome patients being misdiagnosed and suffering from severe complications at an early age. Little is known about the prevalence of Liddle syndrome. Therefore, we aimed to study the prevalence of Liddle syndrome confirmed by genetic testing among young hypertension patients of undetermined causes in China.

METHODS
Three hundred and thirty hypertensive patients of undetermined causes aged 14–40 years who were referred to our hypertension center between January 2010 and December 2014 were enrolled. All patients had their medical histories inquired, blood pressure measured, and blood biochemistry indices analyzed. Patients with hypokalemia (<3.5 mmol/L) underwent genetic testing of the 12th exon of genes encoding β and γ subunits of the epithelial sodium channel (ENaC). Diagnosis was established by identification of mutations that destroy the PY motif of ENaC, and then all family members of the index patient with Liddle syndrome underwent genetic testing and clinical examination.

RESULTS
Among the 330 patients, hypokalemia was found in 48 (14.5%). Of these 48, 5 were diagnosed as Liddle syndrome, yielding a prevalence of 1.52%, besides, 12 of their relatives were identified as well. These Liddle’s patients presented with an earlier onset of hypertension, a stronger family history of hypertension and higher blood pressure than those with essential hypertension. And these Liddle’s patients had hypokalemia and suppressed plasma renin activity.