Am J Transl Res 2023;15(2):1259-1270
www.ajtr.org /ISSN:1943-8141/AJTR0147165

Original Article

A novel GJA5 variant associated with increased risk of essential hypertension

Juan Wang[1*], Xue-Cheng Wang[1*], Zhao-Hua Gu[2*], Guang-Wei Ren[2*], Xiao-Hong Zhao[1], Xin-Kai Qu[3], Ying-Jia Xu[4], Yi-Qing Yang[4,5,6]

[1]Department of Cardiovascular Medicine, East Hospital, Tongji University School of Medicine, Shanghai 200120, China; [2]Sanlin Community Health Service Center, Pudong New District, Shanghai 200124, China; [3]Department of Cardiology, Huadong Hospital, Fudan University, Shanghai 200040, China; [4]Department of Cardiology, Shanghai Fifth People's Hospital, Fudan University, Shanghai 200240, China; [5]Department of Cardiovascular Research Laboratory, Shanghai Fifth People's Hospital, Fudan University, Shanghai 200240, China; [6]Department of Central Laboratory, Shanghai Fifth People's Hospital, Fudan University, Shanghai 200240, China. [*]Equal contributors.

Received October 22, 2022; Accepted January 18, 2023; Epub February 15, 2023; Published February 28, 2023

Abstract: Objectives: Gap junction protein alpha 5 (GJA5), also termed connexin 40 (Cx40), exerts a pivotal role in the mediation of vascular wall tone and two closely-linked polymorphisms in the *GJA5* promoter (-44G>A and +71A>G) have been associated with enhanced susceptibility to essential hypertension (EH) in men. The present investigation aimed to ascertain whether a novel common polymorphism within the upstream regulatory region of *GJA5* (transcript 1B), -26A>G (rs10465885), confers an increased risk of EH. Methods: For this investigation, 380 unrelated patients with EH and 396 unrelated normotensive individuals employed as control persons were enrolled from the Chinese Han-ethnicity population, and their *GJA5* genotypes and plasma renin concentrations were determined by Sanger sequencing and an automated chemiluminescent immunoassay, respectively. The functional effect of the *GJA5* variant was explored in cultured murine cardiomyocytes by dual-light reporter gene analysis. Results: The *GJA5* variant conferred a significantly increased risk for EH (OR: 2.156; 95% CL: 1.661-2.797, $P < 0.0001$), and significantly increased plasma renin levels were measured in patients with EH in comparison with control individuals (46.3±7.2 vs 37.4±6.9, $P < 0.0001$). A promoter-luciferase analysis revealed significantly diminished activity of the promoter harboring the minor allele for this variation in comparison with its wild-type counterpart (165.67±16.85 vs 61.53±8.67, $P = 0.0007$). Conclusions: These findings indicate that the novel variant upstream of the *GJA5* gene (-26A>G) confers a significantly increased vulnerability of EH in humans, suggesting potential clinical implications for precisive prophylaxis and treatment of EH.

Keywords: Essential hypertension, molecular genetics, gap junction protein, GJA5, renin, reporter gene assay

Introduction

Hypertension, also called high blood pressure, remains the most common cardiovascular disease worldwide, with an estimated prevalence of 47% in American adults over 20 years of age (52% for males and 43% for females), which equates to ≈122 million adult persons suffering from hypertension [1]. The prevalence of hypertension increases drastically with increasing age, rising from ≈21% in those 20-34 years old to ≈55% in those 45-55 years old, and up to ≈84% in those ≥ 75 years old [1]. The lifetime risk for hypertension in people aged from 20 to 85 years is > 80% [2]. Hypertension contributes to poor health-correlated quality of life [3-7] and confers a substantially enhanced risk for various complications in multiple target organs, including dysfunction of the vascular endothelium and smooth muscle contractile machinery [8-10], thoracic aortic calcification and aneurysms [11-13], gestational preeclampsia [14-16], chronic kidney disease [17-19], cognitive impairment or dementia [20, 21], ischemic cerebral stroke [22-26], myocardial infarction [27], cardiac structural remodeling and heart failure [28-32], arrhythmias [33], and even for premature cerebrovascular and cardiovascular death [34-38]. In a study of adult subjects 18-30 years old, at baseline, who suffered

hypertension prior to 40 years of age, the incidence of cardiovascular disease was 3.15 per 1000 person-years in those subjects with stage 1 hypertension and 8.04 per 1000 person-years in those subjects with stage 2 hypertension, over an average follow-up of 19 years [39]. In a recent meta-analysis, each 10-mm Hg increase in blood pressure conferred an increased relative risk for cardiovascular disease of 1.25 in females and 1.15 in males, and for cardiovascular mortality of 1.16 in females and 1.17 in males [40]. During the 10-year period between 2009 and 2019, the mortality caused by hypertension increased by 34.2%, and the actual number of deaths resulting from hypertension rose by 65.3% [1]. In contrast, the elimination of hypertension reduced cardiovascular death by 30.4% among male individuals and 38.0% among female individuals [1]. However, in a multi-national study of adult individuals affected with hypertension, ~56% of study individuals knew their diagnosis of hypertension, ~44% were administered with antihypertensive therapy, and ~17% had well-controlled blood pressure [41]. Therefore, hypertension remains a leading contributor to the global morbidity and mortality, giving rise to ≥ 10 million deaths annually [1], which underscores the urgent necessity for identifying the etiology of hypertension.

Although the etiologies of hypertension have not been full elucidated, it is generally understood that hypertension is a multi-factorial complex disease with both non-heritable environmental and heritable pathogenic factors implicated with the pathogenesis of hypertension, including the autonomic nervous system, cardiovascular system, endocrine system, vasopressor/vasodepressor hormones, renal function, body fluid volume, obstructive sleep apnea, pregnancy, and many others [42-48]. In approximately 95% of hypertensive patients who had no recognized causes, such hypertension is termed essential hypertension (EH) caused by the interplay of non-inherited and inherited risk factors, while the remaining 5% of patients are categorized to secondary hypertension, of which about 1% are monogenic diseases [48]. Via pan-genomic scanning with polymorphic genetic markers followed by linkage analysis, several genes were identified to be responsible for hypertension encompassing *APOE*, *NPPA* and *NPPB*, which highlights the crucial roles of these genes in the control of blood pressure [48]. Nevertheless, aggregating evidence demonstrates the polygenic nature of EH in humans, with an estimated heritability of 15%-30% for diastolic blood pressure and 15%-40% for systolic blood pressure, respectively, and indicates that uncommon monogenic diseases merely represent an outermost end of the distribution [48]. Hence recently, investigators have been concentrating on the identification of the genetic polymorphisms associated with increased susceptibility to EH by analysis of candidate genes and genome-wide association study [48]. Up to date, over 1000 genetic loci have been linked to blood pressure, explaining roughly 6% of heritability based on the single nucleotide polymorphism (SNP), though the biological significance of these genetic loci remains largely unclear [48].

Recently, two completely linked SNPs (rs11-552588 +71A>G; rs35594137 -44G>A) in promoter A of the *GJA5* gene, which encodes gap junction protein alpha 5, also termed connexin 40 (Cx40), were related to significantly decreased promoter activity and increased vulnerability to both atrial fibrillation and EH [49-53]. Moreover, a new common SNP (rs10465885 -26A>G) in promoter B of the *GJA5* gene was demonstrated to significantly decrease the expression of Cx40 variant 2 and total Cx40 expression in human atria and was implicated with a significantly enhanced risk for atrial fibrillation [54]. Given the pivotal role of Cx40 in the molecular pathogenesis of EH [52-55], it is justified to make the hypothesis that the common SNP (rs10465885 -26A>G) in promoter B of the *GJA5* gene is associated with a significantly enhanced invulnerability to EH in humans. The major purpose of the work was to ascertain the association of the *GJA5* polymorphism rs10465885 with EH, providing a new molecular target for precision medicine in patients with EH.

Materials and methods

Study participants

This study abides by the principles of the Helsinki declaration. The research protocol was approved by the institutional Ethics Committee of Shanghai Fifth People's Hospital, Fudan University (approval code: 2018-220). Written informed consent for the clinical and genetic

investigations was obtained from each study participant prior to the commencement of the present investigation. For the current research, 380 unrelated patients with EH and 396 unrelated normotensive individuals employed as controls were enlisted from the Chinese Han-ethnicity population in the same geographical area of Shanghai, China. Control people were age- and sex-matched with patients. Blood pressure was measured three times utilizing a standardized mercury sphygmomanometer on the same arm after five minutes of rest in the sitting position by a trained physician blind to the genotypes of the study participants, with the mean of three subsequent measurement values being used in the analysis. In terms of the Chinese guidelines revised in 2018 for the prevention and treatment of hypertension, hypertension was diagnosed with systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of prescribed antihypertensive medications according to the participant's medical records [56]. Normotension was defined as systolic blood pressure < 120 mmHg and diastolic blood pressure < 80 mmHg without taking antihypertensive medications [56]. All study individuals experienced a detailed clinical appraisal, encompassing a review of personal, medical, and familial histories, physical examination, echocardiogram, electrocardiogram, and routine biological assays. The inclusion criteria of this investigation were: 1) all patients with EH met the diagnostic criteria of hypertension, 2) signed informed consent forms to participate in the present investigation, 3) aged 18 years or more, and 4) Han-race Chinese people. The exclusion criteria were: 1) under 18 years of age, 2) other Chinese ethnic minorities, 3) not signed the informed consent form, 4) missing information during the collection of clinical data, and 5) patients with secondary hypertension or with coronary artery disease, primary cardiomyopathy, valvular heart disease, cerebrovascular disease, acute or chronic infection, liver cancer, renal dysfunction, or diabetes mellitus. Blood sample was drawn from each study subject for clinical biochemical analysis and extraction of genomic DNA used for genetic studies.

Genotyping GJA5

Extraction of genomic DNA was implemented from whole blood leucocytes utilizing a genomic DNA purification kit (Qiagen, Germany) as per the manufacturer's procedure protocol and verified on 1.2% agarose gel by electrophoresis. The quality as well as concentration of the purified DNA was assayed utilizing a spectrophotometer (NanoDrop Technologies, USA). The processed DNA was quickly preserved in a refrigerator (Sanyo, Japan) at -80°C until further analysis. The *GJA5* polymorphism of rs10465885 (-26A>G) was genotyped through polymerase chain reaction (PCR)-sequencing of the amplicons from genomic DNA. The genomic DNA sequences of the *GJA5* gene were derived from the GenBank database (accession number: NC_000001.11) at the official website (https://www.ncbi.nlm.nih.gov/nuccore/NC_000001.11?from=147756199&to=147773351&report=genbank&strand=true). The primer pair for amplification of the region encompassing the polymorphism (-26A>G) in the *GJA5* gene (transcript variant B) was designed as follows with the online program Primer3Plus (https://www.primer3plus.com): 5'-CTCTGTCCACAGGCAGGAAG-3' and 5'-CCCTCAAGCTGAGCCTCTTC-3', with the product size being 575 bp. Amplification of genomic DNA fragments by PCR was performed on a PCR instrument (Thermo Fisher Scientific, USA) using the above-mentioned primers and a HotStar Taq DNA polymerase kit (Qiagen, Germany). The total amount of the PCR mixture was equal to 25 μL, including 12.25 μL of distilled deionized water, 5 μL of 5X Q solution, 1 μL of each primer (20 μM), 2 μL of dNTP (2.5 mM each), 1 μL of genomic DNA (100 ng/μL), 2.5 μL of 10X buffer and 0.25 μL (5 U/μL) of HotStar Taq DNA Polymerase (Qiagen, Germany). The PCR program was set as follows: 95°C for 15 min, then 36 thermal cycles of 94°C for 30 sec, 62°C for 30 sec and 72°C for 1 min, with final elongation at 72°C for 8 min. The amplified products were separated via gel electrophoresis, visualized by staining with ethidium bromide and extracted employing a gel extraction kit (Qiagen, Germany). The extracted products underwent sequencing PCR with a cycle sequencing kit (Applied Biosystems, USA). The volume of the sequencing-PCR mixture was equal to 10 μL, containing 4 μL of distilled deionized water, 1 μL of sense or antisense primer (1 μM), 4 μL of Premix, and 1 μL of the isolated amplicons (50 ng/μL). The sequencing-PCR conditions were: 36 thermal cycles of 95°C for 20 sec, 50°C for 15 sec and 60°C for 1 min. The sequencing PCR products

were purified with a PCR isolation kit (Qiagen, Germany) and sequenced on a DNA sequencing apparatus (Applied Biosystems, USA) following the manufacturer's protocol. The sequencing data were used to genotype *GJA5* in the study subjects focusing on the SNP rs10465885.

Biochemical assay

Collection of venous blood specimens and separation of serum/plasma as well as storage of samples, were fulfilled as previously described [56]. Measurements of routine biochemical indicators, including total cholesterol, fasting blood glucose, total triglyceride as well as serum creatinine, were performed using an automated analyzer (Beckman Coulter, USA). The concentration of plasma renin was determined with a renin assay kit (Nichols Institute Diagnostics, USA) as per the manufacturer's protocol.

Construction of recombinant reporter plasmids

As described elsewhere [54], an 859-bp promoter fragment (from -765 bp to +94 bp relative to the first base pair of exon 1B) was amplified by PCR from genomic DNA of the subject homozygous for the G or A allele at the promoter SNP of GJA5 transcript B, using the HotStar Taq DNA Polymerase Kit (Qiagen, Germany) and a specific pair of primers (5'-CTCGCTAGCCTGACCCCATCTTTCCCCATAA-3' and 5'-CTCCTCGAGTTGCTGCCTTGTGTTGTAATCCTC-3'). The A or G allele-containing promoter fragment was doubly digested with the restriction enzymes of NheI (NEB, USA) and XhoI (NEB, USA), separated by gel electrophoresis, isolated utilizing a gel isolation kit (Qiagen, Germany), and then subcloned at the NheI-XhoI sites into a promoter-less pGL3-Basic vector (Promega, USA). The DNA sequences of both the G and A allele-containing recombinant vectors were validated by direct sequencing to ensure that the SNP site in promoter B of GJA5 was the only site of DNA sequence discordance between the two recombinant vectors.

Cell transfection and dual-reporter gene analysis

HL-1 cells (murine atrial cardiomyocytes) were cultured as described previously [54]. HL-1 cells were cultivated in an antibiotic-free medium in 12-well plates for 24 h before transfection with the transfection reagent Lipofectamine 3000 (Invitrogen, USA). Cells were co-transfected with the same amount (1 μg) of either the A or G allele-containing promoter-luciferase plasmid and 1 μg of the pSV-ß-Galactosidase Control Vector (Promega, USA) as an internal control to normalize transfection efficiency. As a negative control, cells were co-transfected with the same amount (1 μg) of the empty pcDNA3.1 plasmid and the control vector. Three independent experiments were executed in triplicate with each expression plasmid. Cells were lysed 48 h later, and the cell lysates were analyzed in triplicate for firefly luciferase and ß-galactosidase activity with a dual-light reporter analysis kit (Thermo Fisher Scientific, USA), and the mean background-subtracted luciferase/ß-galactosidase levels were calculated as the relative promoter activity for each well [54].

Statistical analysis

Normality was evaluated for continuous variables using the Kolmogorov-Smirnov test. Data were presented as means ± standard deviations for continuous variables. Student's unpaired t-test was conducted for the comparison between the two groups. One-way ANOVA with a Tukey-Kramer HSD post hoc test was used to make multiple comparisons. To analyze the categorical variables expressed as numbers and percentages, the Chi-square test was applied. A two-tailed *P*-value of < 0.05 indicated a statistical difference.

Results

Demographical and baseline clinical features of the study subjects

In the current investigation, 380 unrelated patients with EH were clinically assessed in comparison with 396 unrelated normotensive individuals. No significant differences ($P > 0.05$) existed between the hypertensive and normotensive groups in sex, age, body mass index, serum total cholesterol, serum low-density lipoprotein, serum high-density lipoprotein, fast blood glucose, serum triglyceride, serum creatinine, or serum uric acid. However, there were significant differences ($P < 0.05$) observed between the two groups in blood pressure and plasma renin concentration, and the hyperten-

Table 1. Demographical and baseline clinical data of the study subjects

Variable	Hypertensive group (n = 380)	Normotensive group (n = 396)	Statistics
Age (years)	46.8±5.3	47.1±6.1	t = 0.7301; P = 0.4656
Sex (M/F)	202/178	210/186	χ^2 = 0.0013; P = 0.9716
BMI (kg/m^2)	23.6±1.8	23.4±1.6	t = 1.6374; P = 0.1019
SBP (mmHg)	157.2±12.3	117.5±8.4	t = 52.6895; P < 0.0001*
DBP (mmHg)	95.8±7.9	76.4±6.7	t = 36.9453; P < 0.0001*
TC (mmol/L)	4.5±1.0	4.4±0.9	t = 1.4654; P = 0.1432
TG (mmol/L)	1.5±0.7	1.5±0.6	t = 0.0000; P > 0.9999
HDL (mmol/L)	1.2±0.3	1.2±0.4	t = 0.0000; P > 0.9999
LDL (mmol/L)	3.1±0.8	3.0±0.7	t = 1.8552; P = 0.0640
FBG (mmol/L)	4.8±0.9	4.7±0.8	t = 1.6374; P = 0.1019
K^+ (mmol/L)	4.3±0.4	4.3±0.3	t = 1.4654; P = 0.143
Na^+ (mmol/L)	141.8±3.1	140.4±3.5	t = 0.0000; P > 0.9999
SC (μmol/L)	78.6±9.2	77.9±8.4	t = 1.1076; P = 0.2684
SUA (μmol/L)	318.9±27.5	320.1±31.7	t = 0.5623; P = 0.5741
PRC (mIU/L)	46.3±7.2	37.4±6.9	t = 17.5834; P < 0.0001*

Data are presented as mean ± standard deviation or number (percentage) of study subjects. Notes: M: male; F: female; BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; TC: Total Cholesterol; TG: Triglyceride; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; FBG: Fast Blood Glucose; SC: Serum Creatinine; SUA: Serum Uric Acid; PRC: Plasma Renin Concentration. *Significant differences between the hypertensive and normotensive groups (P < 0.05).

sive group had significantly increased diastolic blood pressure, systolic blood pressure, and plasma renin concentration (P < 0.05). The demographical information and baseline clinical data of the study individuals are shown in **Table 1**.

Frequencies of GJA5 alleles and genotypes in hypertensive patients and normotensive individuals

The SNP (rs10465885 -26A>G) in promoter B of the *GJA5* gene was detected in 380 patients with EH and 396 normotensive subjects employed as controls by PCR-sequencing analysis. Consistent with the previous study [54], three genotypes (G/G, A/G and A/A) were observed in both patients and controls. The representative electrophoretic chromatograms exhibiting three genotypes (G/G, A/G and A/A) of the *GJA5* gene (rs10465885) were shown in **Figure 1**.

The frequencies of genotypes and alleles of rs10465885 in promoter B of *GJA5* in EH patients and normotensive control people are given in **Table 2**. As shown in **Table 2**, the frequencies of the A/A, A/G and G/G genotypes for the *GJA5* gene rs10465885 in EH patients were 56.58%, 36.05% and 7.37%, respectively, which in normotensive controls were 75.25%, 22.22% and 2.53%, respectively. There existed a significant difference in the genotypical frequency between the patient and control groups (for A/A: x^2 = 30.18, P < 0.0001; for A/G: x^2 = 18.02, P < 0.0001; for G/G: x^2 = 9.767, P = 0.0009). The frequencies of A and G alleles of the *GJA5* gene rs10465885 in patients with EH were 74.61% and 25.39%, respectively, which in normotensive controls were 86.36% and 13.64%, respectively. There existed significant difference in the frequency of alleles between the EH patient and healthy control groups (x^2 = 34.3, P < 0.0001), and significant association of the G allele of the *GJA5* polymorphism rs10465885 with enhanced risk for EH (odds ratio (OR) = 2.156, 95% confidence limits (CL) = 1.661-2.797, P < 0.0001). Additionally, the genotypes of the *GJA5* polymorphism rs10465885 in EH patients were in conformity with the Hardy-Weinberg equilibrium (χ^2 = 0.8954, P > 0.05), which in normotensive controls were similar (χ^2 = 1.2655, P > 0.05).

Notably, among the 380 EH patients, 72 (18.95%) had electrocardiogram-documented atrial fibrillation, of whom 11 carried A/A alleles, 56 carried A/G alleles, and 15 carried G/G alleles. There existed a significant difference in

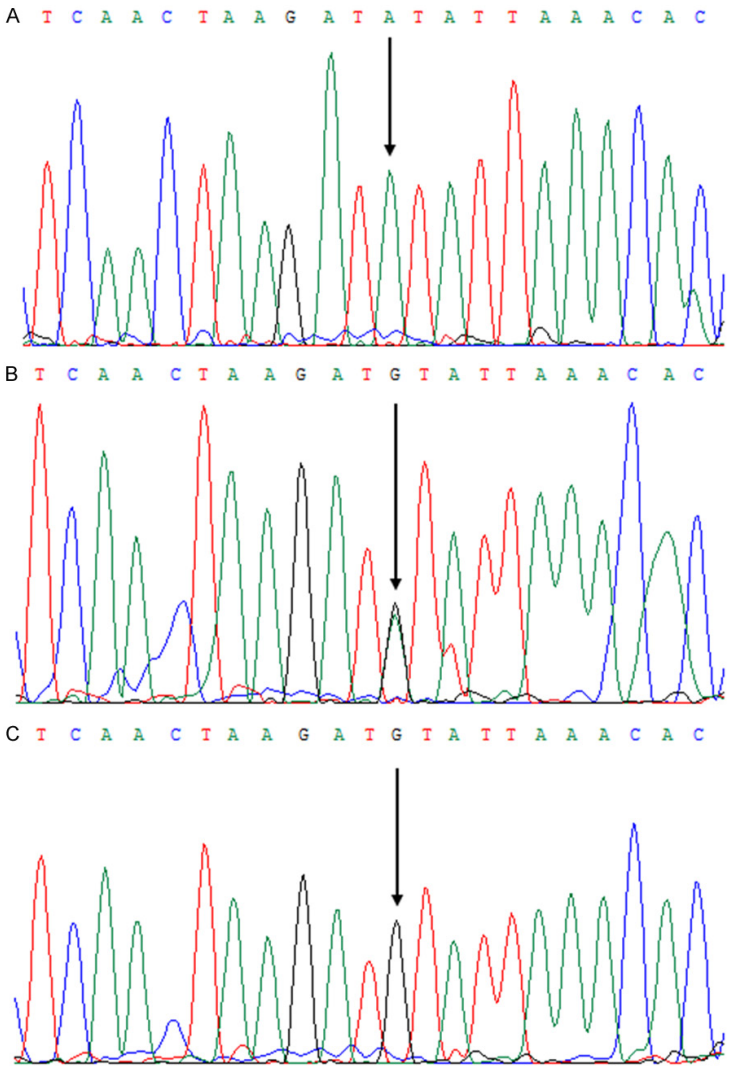

Figure 1. Representative electrophoretic chromatograms displaying the SNP rs10465885 in the *GJA5* gene. A. Representative sequence tracings from an individual homozygous (A/A) for the SNP in promoter B of *GJA5*; B. Representative sequence tracings from an individual heterozygous (A/G) for the SNP in promoter B of *GJA5*; C. Representative sequence tracings from an individual homozygous (G/G) for the SNP in promoter B of *GJA5*.

the incidence of atrial fibrillation between the wild-type genotype and each mutant-type genotype (for A/A vs A/G: $\chi^2 = 69.43$, $P < 0.0001$; for A/A vs G/G: $\chi^2 = 60.88$, $P < 0.0001$). However, no significant difference existed in the incidence of atrial fibrillation between the two mutant-type genotypes (A/G vs G/G: $\chi^2 = 1.529$, $P = 0.2168$).

Association of GJA5 genotypes with plasma renin concentration in hypertensive patients

The plasma renin concentrations of 380 hypertensive patients with different genotypes of the *GJA5* gene rs10465885 are presented in **Table 3**. There existed significant difference in the plasma renin concentration among the EH patients with different genotypes of the *GJA5* gene polymorphism rs10465885 ($F = 2085.41$, $P < 0.0001$). Multiple comparisons were performed between A/A and A/G ($t = 18.4113$, $P < 0.0001$), between A/A and G/G ($t = 68.0408$, $P < 0.0001$), and between A/G and G/G ($t = 44.9372$, $P < 0.0001$).

The variation of GJA5 promoter B reduces promoter activity in transfected cells

As shown in **Figure 2**, HL-1 cells transiently transfected with the reporter plasmid harboring the allele G at the polymorphism site of promoter B displayed 2.69-fold decrease luciferase activity when compared with the HL-1 cells transiently transfected with the vector carrying the A allele, indicating a significant difference (165.6667±16.8471 vs 61.5333±8.6674: $t = 9.51997$, $P = 0.00068$). When comparison among three groups (negative control, A allele and G allele) was performed, similar statistical significance was achieved ($F = 162.32$, $P = 5.975e-06$): for negative control (6.7±1.6) vs A allele (165.6667±16.8471), $t = 158.9667$ and $P < 0.0001$; for negative control (6.7±1.6) vs G allele (61.5333±8.6674), $t = 54.8333$ and $P = 0.00211$; for A allele (165.6667±16.8471) vs G allele (61.5333±8.6674), $t = 104.1333$ and $P = 0.00006$.

Discussion

In the current case-control study, the minor allele of the *GJA5* polymorphism, rs10465885, was first found to confer an enhanced risk of EH in humans and was first associated with an increased plasma renin concentration, suggesting that *GJA5* loss-of-function variation is an alternative molecular pathogenesis of EH, probably by increasing renin release.

Table 2. The frequencies of genotypes and alleles of the GJA5 gene rs10465885 in hypertensive patients and normotensive controls

Group	n	Frequency of genotype			Frequency of allele	
		A/A	A/G	G/G	A	G
EH	380	215	137	28	567	193
NT	396	298	88	10	684	108

Notes: EH: Essential Hypertension; NT: normotension.

Table 3. Plasma renin concentrations of 380 hypertensive patients with different genotypes of the *GJA5* gene polymorphism rs10465885

	A/A (n = 215)	A/G (n = 137)	G/G (n = 28)
PRC (mIU/L)	35.1±5.8	48.3±7.6	125.7±11.2

Note: PRC: Plasma Renin Concentration.

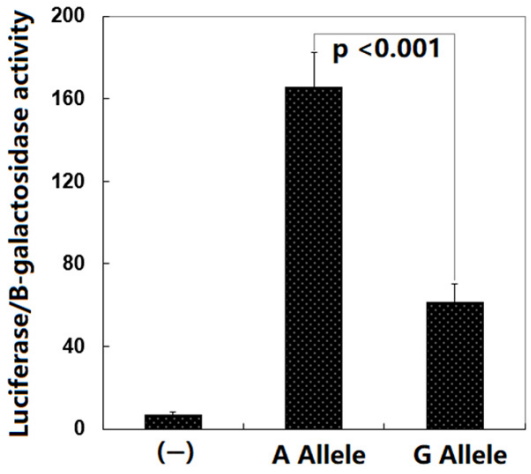

Figure 2. Decreased promoter B activity by the variation of *GJA5*. The ratio of firefly luciferase activity to β-galactosidase activity is given after transient transfection of HL-1 cells with reporter plasmids prompted by the *GJA5* transcript B promoter with G/G or A/A genotype at the promoter B polymorphism, or with a promoter-less vector (-). For each expression plasmid used, three independent experiments were fulfilled in triplicates. Data show the means of the results derived from three experiments. The student's t-test was utilized to compare the two groups (G vs A: t = 8.0540, P = 0.00129).

It is generally understood that the renin-angiotensin-aldosterone system (RAAS) exerts a central role in the mediation of blood pressure [42, 57]. RAAS is a consecutive hormonal framework, which links the renal, adrenal, and cardiovascular system, and functions as a specific hormonal cascade to regulate arterial blood pressure and sodium balance, hence maintaining the homeostasis of fluid and electrolytes [57, 58]. RAAS is not merely confined to systemic circulation but also exists locally in specific tissues encompassing the heart and blood vessels with an action of paracrine [57]. Abnormal RAAS function may contribute to the occurrence of hypertension and the emergence of its associated end-organ damage in the heart, kidney, brain and blood vessels, and genetic variations of the different genes encoding RAAS have been implicated with the susceptibility to EH [57, 58]. Accordingly, inhibition of RAAS has been a main therapeutic strategy for controlling EH and reducing its related organ injuries [59]. Recent investigations have shown that the minor *GJA5* genotypes result in a significant decrease in promoter activity in Cx40-expressing cells of vascular and cardiac origin [52, 54]. Decreased promoter activity might lead to lower Cx40 expression *in vivo*, which could increase the risk for hypertension via the compromised regulation of vasomotor response and control of vascular tone, as supported by the research results from Cx40-knockout mice [52]. Moreover, Cx40 was also involved in the mediation of renin release [60]. Another study revealed that 40Gap27, a Cx40-mimetic peptide, interfered with Cx40-regulated gap-junctional communication by binding the Cx40-docking sites, and in the rats with 40Gap27 administered, decreased renal blood flow and increased arterial blood pressure were observed [61]. Collectively, these results together with the current study indicate that Cx40 exerts a pivotal role in the long-term mediation of blood pressure by affecting renal hemodynamic function.

Gap junctions are an array of intercellular channels, which are constituted by the docking of two hemichannels from a pair of neighbouring cells, with each hemichannel constructed by the assembly of six Cxs [62]. Cx is a transmembrane protein with an intracellular C-terminus, four transmembrane domains, one cytoplasmic loop, two extracellular loops, and an intracellular N-terminus [62]. To date, four distinct isoforms of Cxs, including Cx40, Cx45, Cx43 and Cx37, are discovered to be expressed amply in the endothelial cells and smooth muscles of

blood vessels as well as in the kidney [52]. Exception for Cx37, all these Cxs are also expressed highly in the heart [52]. Gap junction channel connects the plasma membranes of neighboring cells, creating a cell-to-cell pathway, and thereby enabling the direct exchange of ions and small molecules (with molecular mass < 1 kD) between adjacent cells [62]. Besides, under specific conditions such as paracrine signaling or pathological conditions, hemichannels may also functionally serve as transmembrane channels in cells, hence enabling the diffusion of ions as well as small molecules [62]. In addition to playing pivotal roles in the exchange of electrochemical substance between neighboring cells, gap junction channels have also been validated to regulate renin release in the kidney, and regulate vascular reactivity and vasomotor tone, thus are closely related to blood pressure [60, 63].

Association of the polymorphisms of Cx-coding genes with EH has been studied. Firouzi and colleagues [52] genotyped *GJA5* in 191 cases with EH, 198 normotensive subjects, and 178 twin pairs as a healthy control population, and found that two-closely linked polymorphisms of rs35594137 (-44G>A) and rs11552588 (+71A>G) in promoter A of *GJA5* were related to EH in males, but not in females, demonstrating an important contribution of the uncommon alleles or genotypes (-44AA/+71GG) to the increased risk of EH. Additionally, in the control individuals a considerable effect of *GJA5* genotype on systolic blood pressure was observed, with a significantly enhanced systolic blood pressure in women harboring the minor *GJA5* genotypes compared with that in non-carriers [52]. Schmidt and coworkers [53] made a sequencing analysis of exon 1A and exon 2 of *GJA5* in 178 probands (26 suffered from left ventricular hypertrophy, 112 were hypertensive, 29 normotensive, and 11 unknown), and found that the *GJA5* polymorphisms of rs35594137 and rs11552588 were associated with EH as well as left ventricular hypertrophy predominantly in males. Wang and partners [64] made a genetic analysis of the polymorphisms of the genes encoding Cx37 (rs1630310), Cx40 (rs35594137 and rs11552588), and Cx43 (rs1925223) in 1176 subjects (585 cases with EH and 591 normotensive persons as controls) and found that the polymorphisms of *Cx43* rs1925223 and *Cx37* rs1630310 were involved in EH. However, there was no association of the *Cx40* polymorphisms of rs11552588 and rs35594137 with the development of EH. In the present research, the polymorphism rs10465885 in promoter B of *GJA5* was demonstrated to be associated with EH. These findings underscore the key roles of Cxs in the molecular pathogenesis of EH.

It is interesting that the current research reveals a significant association of the polymorphism rs10465885 in *GJA5* with atrial fibrillation in patients with EH. Wirka *et al* [54] previously associated the polymorphism rs10465885 in *GJA5* with lone atrial fibrillation. Moreover, multiple pathogenic mutations in the genes encoding Cx40, Cx43 and Cx45 have been identified to be responsible for atrial fibrillation [65-69]. These findings highlight the critical roles of Cxs in the molecular pathogenesis of cardiac arrhythmias.

Conclusion

In conclusion, the present study firstly indicates that a previously described *GJA5* promoter B polymorphism, rs10465885, confers enhanced susceptibility to EH, and is associated with increased plasma renin concentration. Further investigations with a greater number of samples are necessitated to validate the association of the polymorphism of *GJA5* promoter B with the occurrence of EH. Our current findings suggest potential clinical implications for personalized treatment of EH in a subset of patients.

Acknowledgements

This research was funded by the Key Project of Shanghai Fifth People's Hospital, Fudan University, Shanghai, China (grant number 2018WYZD05), the Chronic Disease Research Program for General Practitioners, Tongji University School of Medicine, Shanghai, China (grant number TYS2018M009), and the National Natural Science Foundation of China (grant number 81770420).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ying-Jia Xu, Department of Cardiology, Shanghai Fifth People's

Hospital, Fudan University, 801 Heqing Road, Shanghai 200240, China. Tel: +86-021-24289511; E-mail: xuyingjia@5thhospital.com; Dr. Yi-Qing Yang, Department of Cardiovascular Research Laboratory, Shanghai Fifth People's Hospital, Fudan University, 801 Heqing Road, Shanghai 200240, China. Tel: +86-021-24289657; E-mail: yangyiqing@fudan.edu.cn

References

[1] Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, Boehme AK, Buxton AE, Carson AP, Commodore-Mensah Y, Elkind MSV, Evenson KR, Eze-Nliam C, Ferguson JF, Generoso G, Ho JE, Kalani R, Khan SS, Kissela BM, Knutson KL, Levine DA, Lewis TT, Liu J, Loop MS, Ma J, Mussolino ME, Navaneethan SD, Perak AM, Poudel R, Rezk-Hanna M, Roth GA, Schroeder EB, Shah SH, Thacker EL, VanWagner LB, Virani SS, Voecks JH, Wang NY, Yaffe K and Martin SS. Heart disease and stroke statistics-2022 update: a report from the American heart association. Circulation 2022; 145: e153-e639.

[2] Chen V, Ning H, Allen N, Kershaw K, Khan S, Lloyd-Jones DM and Wilkins JT. Lifetime risks for hypertension by contemporary guidelines in African American and White men and women. JAMA Cardiol 2019; 4: 455-459.

[3] Peacock E, Joyce C, Craig LS, Lenane Z, Holt EW, Muntner P and Krousel-Wood M. Low medication adherence is associated with decline in health-related quality of life: results of a longitudinal analysis among older women and men with hypertension. J Hypertens 2021; 39: 153-161.

[4] Xiao S, Zhao C, Sun J, Dou Y and Teng M. Effect of high-quality nursing on negative psychological moods and quality of life of elderly patients with hypertension. Am J Transl Res 2021; 13: 3710-3716.

[5] Liao Y, Ye T, Liang S, Xu X, Fan Y, Ruan X and Wu M. Clinical nursing pathway improves disease cognition and quality of life of elderly patients with hypertension and cerebral infarction. Am J Transl Res 2021; 13: 10656-10662.

[6] Pan Y, Ni L, Fang S, Zhang J, Fan W and Shen F. Effect of comprehensive care on the negative emotions and life quality in parturients with postpartum depression and gestational hypertension. Am J Transl Res 2021; 13: 7228-7234.

[7] Ouyang Y, Liu X, He Z and Huang D. Effect of high-quality nursing on postpartum hemorrhage and quality of life in puerperants with gestational hypertension. Am J Transl Res 2022; 14: 304-310.

[8] de Oliveira MG, Nadruz W Jr and Mónica FZ. Endothelial and vascular smooth muscle dysfunction in hypertension. Biochem Pharmacol 2022; 205: 115263.

[9] Yang Q and Hori M. Characterization of contractile machinery of vascular smooth muscles in hypertension. Life 2021; 11: 702.

[10] Li J, Lu Y, Wang Y, Wang X, Kang X, Tang W and Chen L. Long noncoding RNA urothelial carcinoma associated 1 protects human placental vascular endothelial cells from hypoxia-induced damage by regulating the miR-197-3p/histone deacetylase-2 axis in patients with pregnancy-induced hypertension. Am J Transl Res 2022; 14: 6137-6149.

[11] Jan YT, Tsai PS, Longenecker CT, Lin DC, Yun CH, Sung KT, Liu CC, Kuo JY, Hung CL, Wu TH, Lin JL, Hou CJ, Tsai CT, Chien CY and So A. Thoracic aortic calcification and pre-clinical hypertension by new 2017 ACC/AHA hypertension guidelines. Diagnostics 2021; 11: 1027.

[12] Encica S, Molnar A, Manole S, Filan T, Opri□a S, Bursa□iu E, Vulturar R and Damian L. Rare causes of arterial hypertension and thoracic aortic aneurysms-a case-based review. Diagnostics 2021; 11: 446.

[13] Rooprai J, Boodhwani M, Beauchesne L, Chan KL, Dennie C, Wells GA and Coutinho T. Central hypertension in patients with thoracic aortic aneurysms: prevalence and association with aneurysm size and growth. Am J Hypertens 2022; 35: 79-86.

[14] Ardissino M, Slob EAW, Millar O, Reddy RK, Lazzari L, Patel KHK, Ryan D, Johnson MR, Gill D and Ng FS. Maternal hypertension increases risk of preeclampsia and low fetal birthweight: genetic evidence from a mendelian randomization study. Hypertension 2022; 79: 588-598.

[15] Zeng L and Liao C. Multivariate logistic regression analysis of preeclampsia in patients with pregnancy induced hypertension and the risk predictive value of monitoring platelet, coagulation function and thyroid hormone in pregnant women. Am J Transl Res 2022; 14: 6805-6813.

[16] Salazar MR, Espeche WG, Leiva Sisnieguez CE, Minetto J, Balbín E, Soria A, Yoma O, Prudente M, Torres S, Grassi F, Santillan C and Carbajal HA. Nocturnal hypertension and risk of developing early-onset preeclampsia in high-risk pregnancies. Hypertens Res 2021; 44: 1633-1640.

[17] Bae EH, Lim SY, Jung JH, Oh TR, Choi HS, Kim CS, Ma SK, Han KD and Kim SW. Chronic kidney disease risk of isolated systolic or diastolic hypertension in young adults: a nationwide sample based-cohort study. J Am Heart Assoc 2021; 10: e019764.

[18] Khayyat-Kholghi M, Oparil S, Davis BR and Tereshchenko LG. Worsening kidney function is the major mechanism of heart failure in hypertension: the ALLHAT study. JACC Heart Fail 2021; 9: 100-111.
[19] Hall JE, Mouton AJ, da Silva AA, Omoto ACM, Wang Z, Li X and do Carmo JM. Obesity, kidney dysfunction, and inflammation: interactions in hypertension. Cardiovasc Res 2021; 117: 1859-1876.
[20] Hodis JD, Gottesman RF, Windham BG, Knopman DS, Lutsey PL, Walker KA and Alonso A. Association of hypertension according to new American college of cardiology/American heart association blood pressure guidelines with incident dementia in the aric study cohort. J Am Heart Assoc 2020; 9: e017546.
[21] Daugherty AM. Hypertension-related risk for dementia: a summary review with future directions. Semin Cell Dev Biol 2021; 116: 82-89.
[22] He M, Cui B, Wang J, Xiao X, Wu T, Wang M, Yang R, Zhang B, Xu B, He X, Zhang G, Niu X, Li Z, Wang B, Xu B, Hui R and Wang Y. Focus on blood pressure levels and variability in the early phase of acute ischemic stroke with hypertension and carotid stenosis. J Clin Hypertens 2021; 23: 2089-2099.
[23] Wang D, Wang J, Liu J, Qin Y, Lou P, Zhang Y, Zhang Y and Xiang Q. The role of cumulative mean arterial pressure levels in first stroke events among adults with hypertension: a 10-year prospective cohort study. Clin Epidemiol 2022; 14: 665-676.
[24] Kupferman JC, Lande MB, Stabouli S, Zafeiriou, DI and Pavlakis SG. Hypertension and childhood stroke. Pediatr Nephrol 2021; 36: 809-823.
[25] Turana Y, Tengkawan J, Chia YC, Nathaniel M, Wang JG, Sukonthasarn A, Chen CH, Minh HV, Buranakitjaroen P, Shin J, Siddique S, Nailes JM, Park S, Teo BW, Sison J, Ann Soenarta A, Hoshide S, Tay JC, Prasad Sogunuru G, Zhang Y, Verma N, Wang TD and Kario K; HOPE Asia Network. Hypertension and stroke in Asia: a comprehensive review from HOPE Asia. J Clin Hypertens 2021; 23: 513-521.
[26] Song JW, Xiao J, Cen SY, Liu X, Wu F, Schlick K, Li D, Yang Q, Song SS and Fan Z. Sex differences in intracranial atherosclerosis in patients with hypertension with acute ischemic stroke. J Am Heart Assoc 2022; 11: e025579.
[27] Liu X, Song Q, Wu S, Zhou W and Wang X. Prediabetes and risk for myocardial infarction by hypertension status in a Chinese population: a prospective cohort study. J Hypertens 2021; 39: 77-83.
[28] Li X, Guo X, Chang Y, Zhang N and Sun Y. Analysis of alterations of serum inflammatory cytokines and fibrosis makers in patients with essential hypertension and left ventricular hypertrophy and the risk factors. Am J Transl Res 2022; 14: 4097-4103.
[29] Suzuki Y, Kaneko H, Yano Y, Okada A, Itoh H, Matsuoka S, Fujiu K, Yamaguchi S, Michihata N, Jo T, Takeda N, Morita H, Node K, Kim HC, Viera AJ, Oparil S, Yasunaga H and Komuro I. Age-dependent relationship of hypertension subtypes with incident heart failure. J Am Heart Assoc 2022; 11: e025406.
[30] Malek AM, Wilson DA, Turan TN, Mateus J, Lackland DT and Hunt KJ. Incident heart failure within the first and fifth year after delivery among women with hypertensive disorders of pregnancy and prepregnancy hypertension in a diverse population. J Am Heart Assoc 2021; 10: e021616.
[31] Kario K and Williams B. Nocturnal hypertension and heart failure: mechanisms, evidence, and new treatments. Hypertension 2021; 78: 564-577.
[32] Jackson AM, Jhund PS, Anand IS, Düngen HD, Lam CSP, Lefkowitz MP, Linssen G, Lund LH, Maggioni AP, Pfeffer MA, Rouleau JL, Saraiva JFK, Senni M, Vardeny O, Wijkman MO, Yilmaz MB, Saito Y, Zile MR, Solomon SD and McMurray JJV. Sacubitril-valsartan as a treatment for apparent resistant hypertension in patients with heart failure and preserved ejection fraction. Eur Heart J 2021; 42: 3741-3752.
[33] Marazzato J, Blasi F, Golino M, Verdecchia P, Angeli F and De Ponti R. Hypertension and arrhythmias: a clinical overview of the pathophysiology-driven management of cardiac arrhythmias in hypertensive patients. J Cardiovasc Dev Dis 2022; 9: 110.
[34] Malek AM, Wilson DA, Turan TN, Mateus J, Lackland DT and Hunt KJ. Maternal coronary heart disease, stroke, and mortality within 1, 3, and 5 years of delivery among women with hypertensive disorders of pregnancy and prepregnancy hypertension. J Am Heart Assoc 2021; 10: e018155.
[35] Aune D, Huang W, Nie J and Wang Y. Hypertension and the risk of all-cause and cause-specific mortality: an outcome-wide association study of 67 causes of death in the national health interview survey. Biomed Res Int 2021; 2021: 9376134.
[36] Lin Q, Ye T, Ye P, Borghi C, Cro S, Damasceno A, Khan N, Nilsson PM, Prabhakaran D, Ramirez A, Schlaich MP, Schutte AE, Stergiou G, Weber MA, Beaney T and Poulter NR. Hypertension in stroke survivors and associations with national premature stroke mortality: data for 2·5 million participants from multinational screening campaigns. Lancet Glob Health 2022; 10: e1141-e1149.
[37] Hibino M, Otaki Y, Kobeissi E, Pan H, Hibino H, Taddese H, Majeed A, Verma S, Konta T, Yamagata K, Fujimoto S, Tsuruya K, Narita I,

Kasahara M, Shibagaki Y, Iseki K, Moriyama T, Kondo M, Asahi K, Watanabe T, Watanabe T, Watanabe M and Aune D. Blood pressure, hypertension, and the risk of aortic dissection incidence and mortality: results from the J-SCH study, the UK biobank study, and a meta-analysis of cohort studies. Circulation 2022; 145: 633-644.

[38] Vaughan AS, Coronado F, Casper M, Loustalot F and Wright JS. County-level trends in hypertension-related cardiovascular disease mortality-united states, 2000 to 2019. J Am Heart Assoc 2022; 11: e024785.

[39] Yano Y, Reis JP, Colangelo LA, Shimbo D, Viera AJ, Allen NB, Gidding SS, Bress AP, Greenland P, Muntner P and Lloyd-Jones DM. Association of blood pressure classification in young adults using the 2017 American college of cardiology/American heart association blood pressure guideline with cardiovascular events later in life. JAMA 2018; 320: 1774-1782.

[40] Wei YC, George NI, Chang CW and Hicks KA. Assessing sex differences in the risk of cardiovascular disease and mortality per increment in systolic blood pressure: a systematic review and meta-analysis of follow-up studies in the United States. PLoS One 2017; 12: e0170218.

[41] Yang F, Qian D and Hu D; Healthy Aging and Development Study Group, Nanjing Medical University; Data Mining Group of Biomedical Big Data, Nanjing Medical University. Prevalence, awareness, treatment, and control of hypertension in the older population: results from the multiple national studies on ageing. J Am Soc Hypertens 2016; 10: 140-148.

[42] Su C, Xue J, Ye C and Chen A. Role of the central renin-angiotensin system in hypertension (Review). Int J Mol Med 2021; 47: 95.

[43] Zeng X, Ma D, Wu K, Yang Q, Zhang S, Luo Y, Wang D, Ren Y and Zhang N. Development and validation of a clinical model to predict hypertension in consecutive patients with obstructive sleep apnea hypopnea syndrome: a hospital-based study and nomogram analysis. Am J Transl Res 2022; 14: 819-830.

[44] Zhou Z, Deng C and Xiang X. Blood glucose related to pregnancy induced hypertension syndrome. Am J Transl Res 2021; 13: 5301-5307.

[45] Yin X and Yang Z. Efficacy of nifedipine tablets plus aspirin in patients with gestational hypertension and the effect on coagulation function and hemorheology. Am J Transl Res 2021; 13: 7059-7064.

[46] Zhang L, Sun L and Wei T. Correlation between MTHFR gene polymorphism and homocysteine levels for prognosis in patients with pregnancy-induced hypertension. Am J Transl Res 2021; 13: 8253-8261.

[47] Vavřínová A, Behuliak M, Vaněčková I and Zicha J. The abnormalities of adrenomedullary hormonal system in genetic hypertension: their contribution to altered regulation of blood pressure. Physiol Res 2021; 70: 307-326.

[48] Olczak KJ, Taylor-Bateman V, Nicholls HL, Traylor M, Cabrera CP and Munroe PB. Hypertension genetics past, present and future applications. J Intern Med 2021; 290: 1130-1152.

[49] Firouzi M, Ramanna H, Kok B, Jongsma HJ, Koeleman BP, Doevendans PA, Groenewegen WA and Hauer RN. Association of human connexin40 gene polymorphisms with atrial vulnerability as a risk factor for idiopathic atrial fibrillation. Circ Res 2004; 95: e29-e33.

[50] Hauer RNW, Groenewegen WA, Firouzi M, Ramanna H and Jongsma HJ. Cx40 polymorphism in human atrial fibrillation. Adv Cardiol 2006; 42: 284-291.

[51] Juang JM, Chern YR, Tsai CT, Chiang FT, Lin JL, Hwang JJ, Hsu KL, Tseng CD, Tseng YZ and Lai LP. The association of human connexin 40 genetic polymorphisms with atrial fibrillation. Int J Cardiol 2007; 116: 107-112.

[52] Firouzi M, Kok B, Spiering W, Busjahn A, Bezzina CR, Ruijter JM, Koeleman BP, Schipper M, Groenewegen WA, Jongsma HJ and de Leeuw PW. Polymorphisms in human connexin40 gene promoter are associated with increased risk of hypertension in men. J Hypertens 2006; 24: 325-330.

[53] Schmidt K, Kaiser FJ, Erdmann J and de Wit C. Two polymorphisms in the Cx40 promoter are associated with hypertension and left ventricular hypertrophy preferentially in men. Clin Exp Hypertens 2015; 37: 580-586.

[54] Wirka RC, Gore S, Van Wagoner DR, Arking DE, Lubitz SA, Lunetta KL, Benjamin EJ, Alonso A, Ellinor PT, Barnard J, Chung MK and Smith JD. A common connexin-40 gene promoter variant affects connexin-40 expression in human atria and is associated with atrial fibrillation. Circ Arrhythm Electrophysiol 2011; 4: 87-93.

[55] Kurtz A. Connexins, renin cell displacement and hypertension. Curr Opin Pharmacol 2015; 21: 1-6.

[56] Hu J, Shen H, Huo P, Yang J, Fuller PJ, Wang K, Yang Y, Ma L, Cheng Q, Gong L, He W, Luo T, Mei M, Wang Y, Du Z, Luo R, Cai J, Li Q, Song Y and Yang S. Heightened cardiovascular risk in hypertension associated with renin-independent aldosteronism versus renin-dependent aldosteronism: a collaborative study. J Am Heart Assoc 2021; 10: e023082.

[57] Abdel Ghafar MT. An overview of the classical and tissue-derived renin-angiotensin-aldosterone system and its genetic polymorphisms in essential hypertension. Steroids 2020; 163: 108701.

[58] Te Riet L, van Esch JH, Roks AJ, van den Meiracker AH and Danser AH. Hypertension: renin-

angiotensin-aldosterone system alterations. Circ Res 2015; 116: 960-975.
[59] Mullick AE, Yeh ST, Graham MJ, Engelhardt JA, Prakash TP and Crooke RM. Blood pressure lowering and safety improvements with liver angiotensinogen inhibition in models of hypertension and kidney injury. Hypertension 2017; 70: 566-576.
[60] Ryan MJ, Liu B, Herbowy MT, Gross KW and Hajduczok G. Intercellular communication between renin expressing As4.1 cells, endothelial cells and smooth muscle cells. Life Sci 2003; 72: 1289-1301.
[61] De Vriese AS, Van de Voorde J and Lameire NH. Effects of connexin-mimetic peptides on nitric oxide synthase- and cyclooxygenase-independent renal vasodilation. Kidney Int 2002; 61: 177-185.
[62] Guo YH and Yang YQ. Atrial fibrillation: focus on myocardial connexins and gap junctions. Biology 2022; 11: 489.
[63] Means MJ, Pfenniger A, Kwak BR and Delmar M. Regulation of cardiovascular connexins by mechanical forces and junctions. Cardiovasc Res 2013; 99: 304-314.
[64] Wang LJ, Zhang WW, Zhang L, Shi WY, Wang YZ, Ma KT, Liu WD, Zhao L, Li L and Si JQ. Association of connexin gene polymorphism with essential hypertension in Kazak and Han Chinese in Xinjiang, China. J Huazhong Univ Sci Technolog Med Sci 2017; 37: 197-203.
[65] Gollob MH, Jones DL, Krahn AD, Danis L, Gong XQ, Shao Q, Liu X, Veinot JP, Tang AS, Stewart AF, Tesson F, Klein GJ, Yee R, Skanes AC, Guiraudon GM, Ebihara L and Bai D. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. N Engl J Med 2006; 354: 2677-2688.
[66] Sun Y, Yang YQ, Gong XQ, Wang XH, Li RG, Tan HW, Liu X, Fang WY and Bai D. Novel germline GJA5/connexin40 mutations associated with lone atrial fibrillation impair gap junctional intercellular communication. Hum Mutat 2013; 34: 603-609.
[67] Christophersen IE, Holmegard HN, Jabbari J, Sajadieh A, Haunsø S, Tveit A, Svendsen JH and Olesen MS. Rare variants in GJA5 are associated with early-onset lone atrial fibrillation. Can J Cardiol 2013; 29: 111-116.
[68] Thibodeau IL, Xu J, Li Q, Liu G, Lam K, Veinot JP, Birnie DH, Jones DL, Krahn AD, Lemery R, Nicholson BJ and Gollob MH. Paradigm of genetic mosaicism and lone atrial fibrillation: physiological characterization of a connexin 43-deletion mutant identified from atrial tissue. Circulation 2010; 122: 236-244.
[69] Li RG, Xu YJ, Ye WG, Li YJ, Chen H, Qiu XB, Yang YQ and Bai D. Connexin45 (GJC1) loss-of-function mutation contributes to familial atrial fibrillation and conduction disease. Heart Rhythm 2021; 18: 684-693.