Association of AGTR1 gene methylation and its genetic variant in Chinese farmer with hypertension
A case-control study

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Abstract
The objective was to determine the potential associations of the angiotensin II receptor type 1 (AGTR1) gene polymorphism, methylation, and lipid metabolism in Chinese farmers with hypertension.

A case-control study was conducted in Wuzhi county of Henan province in China in 2013 to 2014. A total of 1034 local residents (35–74 years, 386 hypertensive cases, and 648 normotensive subjects) were enrolled in this study. Triglyceride (TG), total cholesterol (TC), high-density lipoprotein, and low-density lipoprotein were measured using automatic chemistry analyzer. The AGTR1 gene promoter methylation level was measured using quantitative methylation-specific polymerase chain reaction method. The single nucleotide polymorphism rs275653 was genotyped with TaqMan probe assay at an applied biosystems platform.

The gender, body mass index (BMI), TG, TC, and family history of hypertension in the hypertension group were significantly higher than those in control group (P < .05). No significant difference was observed in the distribution of AGTR1 rs275653 polymorphism in the hypertension and controls (P > .05). The AGTR1 gene methylation in subjects carrying different genotypes was not significantly different (P > .05). The logistic regression analysis found the AGTR1 gene methylation level was negative correlation with hypertension in the present study (odds ratio, 0.946, 95% confidence interval, 0.896–0.999) through adjusting for age, gender, BMI, education, smoking, alcohol drinking, fruit and vegetable intake, pickles intake, and family history of hypertension.

The association of AGTR1 gene hypomethylation and essential hypertension was observed in Chinese farmers; no significant difference was observed in the distribution of AGTR1 rs275653 polymorphism.

Abbreviations: AGTR1 = angiotensin II receptor type 1, DBP = diastolic blood pressure, HDL = high-density lipoprotein, LDL = low-density lipoprotein, RAS = renin-angiotensin system, SBP = systolic blood pressure, TC = total cholesterol, TG = triglyceride.

Keywords: AGTR1 gene, DNA methylation, hypertension, lipid metabolism

1. Introduction
Hypertension is a primary risk factor in cardiovascular systems,[1,2] increasing prevalence rate both in developed and developing countries.[3,4] The number of people who suffer from hypertension is estimated to be about 1.5 billion all over the world by 2025.[5] Essential hypertension mainly involves interaction between genetic and environmental factors.[6] Alterations in genetic or environmental factors may contribute to the pathogenesis of essential hypertension. Several genetic variations involved in pathogenesis of hypertension have been found, including single nucleotide polymorphisms of rs4343 and rs4351 in the angiotensin I converting enzyme gene, rs7194256 in the ETS transcription factor ETS domain-containing protein (ELK3) gene, and rs1042039, rs1054889, and rs2073316 in the xanthine dehydrogenase gene.[7]

The renin-angiotensin system (RAS) is a vital humoral regulation system, which is related to the adjustment of blood pressure.[8] Angiotensin II is a major effector peptide in RAS, leading to vasoconstriction and water-sodium retention.[9] Angiotensin II receptor plays a critical role in mediating the RAS pathway, helping to manage the blood pressure and fluid balance.[10]
volume.[10] Angiotensin II receptor type 1 (AGTR1) and type 2 (AGTR2) are subtypes of AGTR, and it is documented that the AGTR1 gene is extremely associated with the etiology of hypertension and other cardiac outcomes.[11] The AGTR1 gene, located on chromosomes 3q21 to 25 and spanning >55 kb, is composed of 5 exons, with the first 4 encoding the 5'-untranslated region and the fifth being the coding region.[12] Recently, the studies on genetic polymorphisms have reported that the single nucleotide polymorphism (SNP) of the AGTR1 gene is related to the pathogenesis of hypertension.[13] However, few studies on the relationship between epigenetic markers like DNA methylation, another important regulating factor in gene expression, and hypertension were reported,[14] and whether the epigenetic modifications of the AGTR1 gene were associated with hypertension still remains unclear. In fact, studies on metabolism-related diseases showed that DNA methylation can affect lipid metabolism and lead to metabolic diseases, including gestational diabetes mellitus, child obesity, and hypercholesterolemia.[15,16]

Therefore, a case-control study was conducted in rural areas of China, to explore whether genetic variant and DNA methylation status are related to lipid metabolism, even hypertension.

2. Methods

2.1. Location and Population

Based on previous study, 3 administrative villages in Wuzhi county of Henan province were selected as the investigated sites using random sampling method from 2013 to 2014. Local farmers aged 35 to 74 years who lived in the county for >5 years were considered as subjects by cluster sampling. Participants with following conditions were excluded: a) stress-induced hypertension or high altitude hypertension; b) kidney and liver transplantation or dysfunction; c) cranioencebral injuries; d) endocrine disorders; e) malignant tumor or other severe systemic diseases; f) mental illness and anxiety; g) women in gestation or lactation period, or child-bearing period without contraception; h) substance abuse. A total of the 427 hypertension cases and 717 normotensive people were selected according to the Chinese guidelines on prevention and treatment of hypertension and other cardiac outcomes.[11] The dyslipidemia was defined as the presence of ≥1 abnormal serum lipid concentrations according to the Chinese guidelines on prevention and treatment of dyslipidemia in adults, or usage of antidyislipidemia medications in the past 2 weeks.

2.2. Diagnosis for Hypertension

Blood pressure of each individual was measured in sitting position using electronic sphygmomanometer (Omron HEM-770AFuzzy, Kyoto, Japan) by trained operators. The systolic blood pressure (SBP) and diastolic blood pressure (DBP) of every single participant was measured 3 times, with an interval of half minute, then the average value was calculated. Before each measurement, subjects were at resting status for at least five minutes. Hypertensive patients were defined as SBP ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg.

2.3. Questionnaires and Biological Samples Collection

An in-person interview was performed at the village clinics using a standardized and structured questionnaire to collect demographic characteristics, individual behavior (such as diet, smoking, and alcohol consumption), occupational, medical conditions, and medication use. A total of 10 mL of fasting blood was collected from each subject, with 5 mL anticoagulation and 5 mL nonanticoagulation. After centrifugation within 2h, serum and white blood cells were separated and frozen at -80 °C for subsequent analyses. The triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) in serum were measured by Hitachi-7080 automatic biochemical analyzer (Hitachi Ltd., Tokyo, Japan) according to the manufacturer’s protocols. Each sample was run in duplicate, and 15% to 20% of total samples were retested randomly. The intra- and inter-assay coefficients of variation were <10% for these assays. The dyslipidemia was defined as the presence of ≥1 abnormal serum lipid concentrations according to the Chinese guidelines on prevention and treatment of dyslipidemia in adults, or usage of antidyislipidemia medications in the past 2 weeks.

2.4. Determination of AGTR1 Methylation Level

Genomic DNA was extracted using whole blood genomic DNA extraction kits with paramagnetic particle method (BioTeke Corporation, Beijing, China). Bisulfate modification of genomic DNA was performed by using the EZ DNA Methylation-Gold Kit (Zymo Research, CA, USA). The AGTR1 gene methylation was performed by quantitative methylation-specific polymerase chain reaction (PCR) method (Agilent MX3000P, Santa Clara, CA, USA). The sequence in promoter region of AGTR1 gene was searched using University of California Santa Cruz (UCSC)/Ensembl (http://genome.ucsc.edu/) and the methylated specific primers were designed by MethPrimer v1.0 (http://www.urogene.org/cgi-bin/methprimer/methprimer.cgi). Two pairs of PCR primers were used to conduct methylation-specific PCR (methylated specific primers: L, 5'-AATGTGGAGAATACTATTTCTCTGT-3'; R, 5'-CTCTCCCTCTGAAATATTAACAC-3'; unmethylated specific primers: L, 5'-AATGTGGAGAATACTATTTCTCTGT-3'; R, 5'-CTCTCCCTCTGAAATATTAACAC-3'). PCR amplification was performed in a 20.0 μL reaction mixture with the following concentrations: 7.5 μL of 2x Power SYBR Green PCR Master Mix (Applied Biosystems, Cheshire, United Kingdom), 1.5 μL primer with a concentration of 1.25 μm/L, and 60.0 ng of bisulfite-treated DNA template with approximate concentration of 100.0 pg/mL. PCR conditions were as follows: predenaturation at 95°C for 10 minutes, 40 cycles for degeneration at 95°C for 15 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds. Negative controls were set for each experiment. Each sample was run in duplicate, and 15% of total samples were retested randomly. The intra- and inter-assay coefficients of variations <10% were considered being qualified. The rate of DNA methylation was calculated using the reference method.

2.5. Genotyping

The AGTR1 gene was genotyped at the rs275653 site in promoter region with TaqMan probe assay using the Applied Biosystems platform (ABI 7500 Fast Real-Time PCR system, Foster City, USA). The primers and probes for SNPs were designed by Applied Biosystems Inc (Applied Biosystems, Cheshire, United Kingdom), and the allelic discrimination was detected automatically using Sequence Detection Systems 2.1 software on the 7500 Fast Real-Time PCR system. Real-time PCR reaction was carried out in a 12.0 μL volume using 0.1 μL TaqMan probe, 6.0 μL Mix, and 100 ng template DNA. Amplification was obtained by predenaturation at 95°C for 10 minutes, 40 cycles for degeneration at 95°C for 15 seconds, and annealing at 60°C for 60 seconds.
2.6. Statistical Analysis

The database was established using the Epidata 3.0 software (Epidata Association Odense, Denmark) and all the data was double entered into the database by different operators. Differences in age, body mass index (BMI), TG, TC, HDL, LDL, and the AGTR1 methylation level between 2 groups were examined by independent sample t tests. A Chi-square test was used to evaluate the differences in gender, education, the prevalence of alcohol drinking and smoking, fruit and vegetable intake, pickles intake, and family history of hypertension. The binary logistic regression considered hypertension as dependent variable, in which methylation level, TC, TG, HDL, LDL, and SNP of rs275653 were included as covariates. Data were presented as mean ± standard deviation. The P < .05 was considered statistically significant. All analyses were performed using the SPSS 21.0 (SPSS Inc., Chicago, USA).

3. Results

3.1. General Characteristics of Included Subjects

As shown in Table 1, a total of 1034 local farmers were enrolled in the study, with 386 hypertension and 648 control subjects. The gender (male/female), BMI, SBP, DBP, TG, and TC in the hypertension group were significantly higher than those in the control group (P < .05). The prevalence of family history of hypertension was 43.5% in hypertension and 34.1% in controls, with significant difference between the 2 groups. No significant differences on education, smoking, drinking, fruit and vegetable intake, pickles intake, HDL, LDL, and AGTR1 methylation were observed in the 2 groups (P > .05).

3.2. Genotype Distribution of AGTR1 rs275653

Table 2 showed the AGTR1 genotype frequencies of locus rs275653 in the hypertension and control groups. All the SNP genotype frequencies in control group were consistent with the Hardy-Weinberg equilibrium (χ² = 2.314, P = .314). No significant difference was observed in the distribution of AGTR1 rs275653 polymorphism in the hypertension and controls (P > .05). Subjects carrying AG/GG genotypes of rs275653 did not increase the risk of essential hypertension compared with subjects carrying the AA genotype.

3.3. Association Between AGTR1 rs275653 Genotypes and DNA Methylation

The levels of TG, TC, HDL, LDL, and DNA methylation stratified by genotypes were summarized in Table 3. It turned out that no significant results were observed for the above biomarkers among different farmers carrying different genotypes of AGTR1 rs275653 (P > .05, respectively).

3.4. The Effect of AGTR1 Promoter Region Methylation on the Risk of Hypertension

We further analyzed the lipid metabolism and genetic risk factors of hypertension using binary logistic regression, in which AGTR1 rs275653 genotypes, methylation level, TC, TG, HDL, and LDL were included as covariates (Table 4). It turned out that the AGTR1 promoter region methylation level (odds ratio, 0.946, 95% confidence interval, 0.896–0.999) was a protective factor of hypertension in the present study (P = .047).

4. Discussion

Our findings suggest a significant association between the AGTR1 gene methylation and hypertension. However, the AGTR1 rs275653 locus polymorphism may not be a valuable genetic marker for differential risk of essential hypertension among Chinese farmers.

The RAS is associated with the onset of various cardiovascular diseases.[17,18] The AGTR1 gene is a key gene of RAS system and closely related to the occurrence and development of hypertension. However, few studies on the relationship between the AGTR1 gene methylation and hypertension. In the present study,

| Table 1 | General characteristics of included population (n = 1034). |
|---|---|
| Characteristics | Hypertension (n = 386) | Controls (n = 648) | χ² | P |
| Age (year) | 54.99 ± 9.12 | 55.19 ± 9.81 | −0.303 | .762 |
| Gender | | | 4.922 | .027 |
| Male | 211 (54.7) | 308 (47.5) | | |
| Female | 215 (53.3) | 340 (51.5) | | |
| BMI (kg/m²) | 26.43 ± 3.79 | 25.31 ± 3.41 | 4.921 | <.001 |
| SBP (mm Hg) | 145.75 ± 14.13 | 120.01 ± 10.34 | 31.177 | <.001 |
| DBP (mm Hg) | 91.93 ± 8.68 | 76.56 ± 7.05 | 29.463 | <.001 |
| Triglyceride | 11.93 ± 4.86 | 7.56 ± 7.05 | 29.463 | <.001 |
| Total cholesterol | 1.96 ± 1.48 | 1.75 ± 1.37 | 2.346 | .019 |
| High-density lipoprotein | 4.69 ± 0.96 | 4.56 ± 0.97 | 1.304 | .193 |
| Low-density lipoprotein | 1.23 ± 0.29 | 1.24 ± 0.31 | −0.568 | .570 |
| AGTR1 methylation (%) | 3.07 ± 2.28 | 3.33 ± 2.90 | −1.479 | .140 |
| Education | | | | |
| ≤ Primary school | 147 (38.2) | 293 (45.4) | | |
| Junior high school | 175 (45.5) | 275 (42.6) | | |
| ≥ Senior high school | 63 (16.4) | 78 (12.1) | | |
| Smoking | | | | |
| Yes | 143 (37.0) | 224 (34.6) | | |
| No | 243 (63.0) | 424 (65.4) | | |
| Alcohol drinking | | | | |
| Yes | 81 (21.0) | 125 (19.3) | | |
| No | 305 (79.0) | 523 (80.7) | | |
| Fruits and vegetables intake | | | | |
| ≥ 500 g/d | 131 (34.0) | 207 (31.9) | | |
| < 500 g/d | 254 (66.0) | 441 (68.1) | | |
| Pickles intake* | | | | |
| ≥ 2 g/d | 121 (31.4) | 236 (38.6) | | |
| < 2 g/d | 264 (68.6) | 408 (63.2) | | |
| Family history of hypertension | | | | |
| Yes | 168 (43.5) | 221 (34.1) | | |
| No | 218 (56.5) | 427 (65.9) | | |

Values in bold are significant at P < .005.

AGTR1 = angiotensin II receptor type 1, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure.

*Pickles indicate the additional salt intake except for salt in daily diet.

| Table 2 | Genotype and allele frequencies of polymorphisms across AGTR1 rs275653 between hypertension and control group. |
|---|---|
| AGTR1 rs275653 | Hypertension, n (%) | Controls, n (%) | P | OR (95% CI) |
| Genotype | 297 (78.0) | 490 (77.0) | — | — |
| AA | 81 (21.3) | 141 (22.2) | .734 | 1.06 (0.78–1.44) |
| AG | 3 (0.8) | 5 (0.8) | .989 | 1.01 (0.24–4.26) |
| GG | 675 (88.9) | 1211 (88.5) | — | — |
| Allele | 84 (11.1) | 146 (11.5) | .754 | 1.05 (0.79–1.39) |

AGTR1 = angiotensin II receptor type 1, G = confidence interval, OR = odds ratio.
there was significant association between the AGTR1 gene promoter region methylation and hypertension. Meanwhile, Fan et al. identified a significantly lower CpG1 methylation level in the AGTR1 gene promoter region in essential hypertension cases than in controls. Lin et al. identified a significantly lower CpG1 methylation level in essential hypertension. It suggested that lower methylation level of individuals with AA genotype were more susceptible to suffer from hypertension. Lin et al. used melting temperature (Tm) parameters; therefore, our results were entirely trustworthy. It is well known that many risk factors for hypertension and their mechanisms of action and risk intensity are also different. The study of these factors is limited by the research population, sample size, methods, and other conditions. Therefore, future studies should be based on mechanism research and take into consideration the details of the combined effects of genes and environment involved in the RAS system on essential hypertension.

In conclusion, the hypomethylation of AGTR1 gene promoter region is associated with the hypertension, and no significant difference was observed in the distribution of AGTR1 rs275653 polymorphism.

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Author contributions

Zhi-yuan Li, Qiang Ma, and Fang-fang Yu participated in the study design, performed the experiments, analyzed the data, and drafted the article. Yue Ba and Fang-fang Yu provided funding, designed and supervised the study. Qiang Ma, Xing Li, Shui-yuan Yu, and Juan Zuo recruited study participants, collected the samples, and performed DNA methylation examination. Chong-jian Wang and Wen-jie Li contributed with scientific input to the design of the experiment. All authors participated in the article editing and approved the final article.

References

[1] Armani C, Botto N, Andreassi MG, et al. Molecular markers of cardiovascular damage in hypertension. Curr Pharm Des. 2013;19:2341–50.
[2] Sun Z. Aging, arterial stiffness, and hypertension. Hypertension. 2015;66:252–6.
[3] Zheng L, Dai Y, Fu P, et al. Secular trends of hypertension prevalence based on 2017 ACC/AHA and 2018 Chinese hypertension guidelines: results from CHNS data (1991-2015). J Clin Hypertens (Greenwich). 2021;23:28–34.
[4] Poulter NR, Prabhakaran D, Caulfield M. Hypertension. 2021;23:801–12.
[5] Kearney PM, Whelton M, Reynolds K, et al. Global burden of hypertension: analysis of worldwide data. Lancet. 2005;365:217–23.
[6] Bigazzi R, Zagato I, Lanzani C, et al. Hypertension in high school students: genetic and environmental factors: the HYGEF study. Hypertension. 2020;75:71–8.
[7] Wang N, Li X, Zhang Q, et al. Association of angiotensin-converting enzyme gene polymorphism with pulse pressure and its interaction with obesity status in Heilongjiang province. Clin Exp Hypertens. 2019;41:70–4.
[8] Gupta S, Chattopadhaya I, Agrawal BK, et al. Correlation of renin angiotensin system (RAS) candidate gene polymorphisms with response to Ramipril in patients with essential hypertension. J Postgrad Med. 2015;61:21–6.

Table 3

Lipid metabolism and DNA methylation status in farmers carrying different genotypes.

| Markers | AA | AG | GG | F | P | Control group |
|---|---|---|---|---|---|---|
| TG (mmol/L) | 2.00 ± 1.54 | 1.76 ± 1.17 | 1.47 ± 1.59 | 1.00 | .37 | 1.72 ± 1.32 | 1.83 ± 1.48 | 2.33 ± 2.71 | 0.76 |
| TC (mmol/L) | 4.69 ± 0.99 | 4.69 ± 0.87 | 4.28 ± 0.79 | 0.27 | .76 | 4.56 ± 0.96 | 4.61 ± 1.05 | 4.82 ± 0.89 | 0.31 |
| HDL (mmol/L) | 1.23 ± 0.28 | 1.22 ± 0.30 | 1.41 ± 0.47 | 0.50 | .56 | 1.24 ± 0.30 | 1.25 ± 0.32 | 1.32 ± 0.51 | 0.21 |
| LDL (mmol/L) | 2.59 ± 0.74 | 2.41 ± 0.71 | 2.20 ± 0.83 | 1.18 | .31 | 2.55 ± 0.72 | 2.56 ± 0.82 | 2.88 ± 0.50 | 0.40 |
| Methylation (%) | 3.11 ± 2.23 | 2.90 ± 2.48 | 4.71 ± 2.48 | 1.02 | .36 | 3.46 ± 3.07 | 2.92 ± 2.28 | 1.64 ± 1.10 | 2.73 |

HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglyceride.

Table 4

Association of AGTR1 gene polymorphism, methylation and lipid metabolism with hypertension using the logistic regression analysis.

| β | SE | Wald χ² | P | OR (95% CI) |
|---|---|---|---|---|
| AGTR1 rs275653/AA | — | — | 0.537 | .765 | — | — |
| AGTR1 rs275653/AG | —0.035 | 0.169 | 0.043 | .836 | 0.965 (0.693–1.346) | — |
| AGTR1 rs275653/GG | 0.542 | 0.783 | 0.479 | 1.719 (0.371–7.976) | — |
| AGTR1 methylation | —0.056 | 0.028 | 3.952 | .047 | 0.046 (0.896–0.999) | — |
| TG (mmol/L) | 0.084 | 0.109 | 0.591 | .442 | 1.088 (0.878–1.348) | — |
| TC (mmol/L) | 0.119 | 0.101 | 1.381 | 2.40 | 1.127 (0.923–1.374) | — |
| HDL (mmol/L) | —0.181 | 0.337 | 0.287 | 0.502 | 0.835 (0.431–1.617) | — |

Values in bold are significant at P < 0.005. Adjusted for age, gender, body mass index, education, smoking, alcohol drinking, fruit and vegetable intake, pickles intake, and family history of hypertension. AGTR1 = angiotensin II receptor type 1, CI = confidence interval, HDL = high-density lipoprotein, OR = odds ratio, TC = total cholesterol, TG = triglyceride.
[9] Giani JF, Janjulia T, Taylor B, et al. Renal generation of angiotensin II and the pathogenesis of hypertension. Curr Hypertens Rep. 2014;16:477.

[10] Akazawa H, Yano M, Yabumoto C, et al. Angiotensin II type 1 and type 2 receptor-induced cell signaling. Curr Pharm Des, 2013;19:2988–95.

[11] Holmes LJ, Lim A, Comeaux CR, et al. DNA methylation of candidate genes (ACE II, IFN-γ, AGTR 1, CKG, ADD1, SCNN1B and TLR2) in essential hypertension: a systematic review and quantitative evidence synthesis. Int J Environ Res Public Health. 2019;16:4829.

[12] Chen K, Xiao P, Li G, et al. Distributive characteristics of the CYP2C9 and AGTR1 genetic polymorphisms in Han Chinese hypertensive patients: a retrospective study. BMC Cardiovasc Disord. 2021;21:73.

[13] Kim HK, Lee H, Kwon JT, et al. A polymorphism in AGT and AGTR1 gene is associated with lead-related high blood pressure. J Renin Angiotensin Aldosterone Syst. 2015;16:712–9.

[14] Guo W, Han J, Wu S, et al. Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate affects lipid metabolism in zebrafish larvae via DNA methylation modification. Environ Sci Technol. 2020;54:355–63.

[15] Houde AA, St-Pierre J, Hivert MF, et al. Placental lipoprotein lipase DNA methylation levels are associated with gestational diabetes mellitus and maternal and cord blood lipid profiles. J Dev Orig Health Dis. 2014;5:132–41.

[16] Deodati A, Inzaghi E, Liguori A, et al. IGF2 methylation is associated with lipid profile in obese children. Horm Res Paediatr. 2013;79:361–7.

[17] de Kloet AD, Liu M, Rodriguez V, et al. Role of neurons and glia in the CNS actions of the renin-angiotensin system in cardiovascular control. Am J Physiol Regul Integr Comp Physiol. 2015;309:R444–58.

[18] Vinturache AE, Smith FG. Angiotensin type 1 and type 2 receptors during ontogeny: cardiovascular and renal effects. Vascul Pharmacol. 2014;63:145–54.

[19] Fan R, Mao S, Zhong F, et al. Association of AGTR1 promoter methylation levels with essential hypertension risk: a matched case-control study. Cyto.genet Genome Res. 2015;147:95–102.

[20] Lin J, Lin S, Wu Y, et al. Hypomethylation of the angiotensin II type 1 receptor (AGTR1) gene along with environmental factors increases the risk for essential hypertension. Cardiology. 2017;137:126–35.

[21] Laurens HJ, Andrew L, Camilla BC, et al. DNA methylation of candidate genes (ACE II, IFN-γ, AGTR 1, CKG, ADD1, SCNN1B and TLR2) in essential hypertension: a systematic review and quantitative evidence synthesis. Int J Environ Res Public Health. 2019;16:4829.

[22] Gonzalez J, Valls N, Brito R, et al. Essential hypertension and oxidative stress: new insights. World J Cardiol. 2014;6:353–66.

[23] Tsounis D, Bouras G, Giannopoulos G, et al. Inflammation markers in essential hypertension. Med Chem. 2014;10:672–81.

[24] Krüger N, Biwer LA, Good ME, et al. Loss of endothelial FTO antagonizes obesity-induced metabolic and vascular dysfunction. Circ Res. 2020;126:232–42.

[25] Lin S, Liu B, Wu C, et al. Interaction between occupational stress and GR gene polymorphisms on essential hypertension among railway workers. J Occup Health. 2014;55:349–58.

[26] Gatti RR, Santos PS, Sena AA, et al. The interaction of AGT and NOS3 gene polymorphisms with conventional risk factors increases predisposition to hypertension. J Renin Angiotensin Aldosterone Syst. 2013;14:360–8.