Correlation between single nucleotide polymorphisms in the 3 primer untranslated region of PTX3 and the risk of essential hypertension

A case–control study

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Abstract
The aim of this study was to investigate the correlation between single-nucleotide polymorphisms (SNPs) in the 3 primer of untranslated region (3′UTR) of the Pentraxin 3 (PTX3) gene and the risk of essential hypertension (EHT).

PTX3 genotypes, rs2614, rs111451363, and rs73158510 locus, were found in 260 patients with EHT and 260 healthy controls. Quantitative real-time polymerase chain reaction was used to detect plasma hsa-miR-4766-5p levels. Enzyme-linked immunosorbent assay was used to detect plasma PTX3 levels. The dual-luciferase reporter assay was used to identify the binding site of hsa-miR-4766-5p to the PTX3.

PTX3 rs2614 locus T allele was a high risk factor for EHT (odds ratio [OR]=2.76, 95% confidence interval [CI]: 1.86–4.09, P < .01). Sex and diabetes history affected the correlation between PTX3 gene rs2614 locus SNP and EHT risk. The CCG haplotype was a protective factor for EHT (OR=0.40, 95% CI: 0.28–0.67, P < .01), whereas the TCG haplotype was a risk factor for EHT (OR=2.35, 95% CI: 1.51–3.66, P < .01). The plasma PTX3 level of patients with EHT was significantly higher than that of the control group, and the difference was statistically significant (P < .01). The area under the curve for the diagnosis of EHT according to the plasma PTX3 level was 0.62 (95% CI: 0.57–0.66, P < .01). The plasma hsa-miR-4766-5p level in patients with EHT was significantly lower than that in the control group (P < .01). The area under the curve for the diagnosis of EHT according to the plasma hsa-miR-4766-5p level was 0.88 (95% CI: 0.85–0.91, P < .01). Plasma PTX3 levels were significantly negatively correlated with hsa-miR-4766-5p levels in patients with EHT and the control group (r = −0.87, −0.85, P < .01, P < .01). The PTX3 gene rs2614 locus C allele was the target gene of hsa-miR-4766-5p.

The PTX3 rs2614 locus SNP is significantly associated with EHT risk.

Abbreviations: BMI = body mass index, CI = confidence interval, CVC = cross-validation consistency, EHT = essential hypertension, EILSA = Enzyme-linked immunosorbent assay, NCBI = National Center for Biotechnology Information, OR = odds ratio, PCR = polymerase chain reaction, PTX3 = Pentraxin 3.

Keywords: essential hypertension, microRNA, PTX3, single-nucleotide polymorphism

1. Introduction
Chronic history of hypertension is a common cause of renal failure, myocardial infarction, stroke, heart failure, and even death.[1,2] Essential hypertension (EHT) is the most common type of hypertension. Studies have shown that EHT is the result of a combination of genetic and environmental factors.[3–5] EHT is a traditional risk factor for atherosclerosis, targeting the organ closely related to the corresponding blood vessel damaged in atherosclerosis.[6,7] Atherosclerosis is considered as an immune-
mediated inflammatory process.\textsuperscript{[8,9]} Pentraxin 3 (PTX3) is the earliest identified long normal pentameric protein and is a highly conserved family of orthomeric proteins with short normal pentameric protein C.\textsuperscript{[10,11]} It plays an important role in innate immunity and inflammatory response and is closely related to the occurrence and development of atherosclerosis. It can also play a cardiovascular protective role by balancing immune inflammation.\textsuperscript{[12,13]} Related research showed that PTX3 may be an important indicator of cardiovascular inflammation and injury.\textsuperscript{[14,15]} At the same time, it plays a role in the innate immune response and inflammation of the kidney. Normal human kidneys and proximal glomerular epithelial cells have PTX3 expression, and its levels are associated with urinary protein and endothelial cell function. The relationship between pentameric protein C and hypertension has been confirmed by related researches; however, PTX3 is currently less studied in patients with hypertension. Because of the common pathophysiological process of atherosclerosis and because macrophages and endothelial cells are the main sources of PTX3, PTX3 level may better reflect the inflammatory status of hypertension vascular beds, which may represent a subclinical EHT, an important marker of arteriosclerosis and early kidney damage.

In this study, we selected 3 SNP loci with a 3'UTR minor allele frequency of >0.01 of PTX3 according to the Variation Viewer (https://www.ncbi.nlm.nih.gov/variation/view/). The proportion of individuals with hypertension in China is high, and different genetic backgrounds should be considered in clinical research and given sufficient attention. We used a case-control study to analyze the correlation between SNPs at rs2614, rs111451363, and rs73158510 of PTX3 gene and the EHT risk, and to provide a reference for the prevention and treatment of EHT.

2. Materials and methods

2.1. Ethics statement

The research was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the ethics committee of Puijiang Branch of the First Affiliated Hospital, School of Medicine, Zhejiang University and all subjects signed an informed consent form.

2.2. Participants

From August 2017 to August 2019, 260 patients with EHT were enrolled as research participants in Puijiang Branch of the First Affiliated Hospital, including 127 men and 133 women, aged 35 to 81 years (mean, 54.88 ± 7.86 years). The diagnostic criteria of EHT are in accordance to 2010 Chinese guidelines for the management of hypertension.\textsuperscript{[16]} Exclusion criteria are as follows: history of malignancy; secondary hypertension; history of severe liver and kidney diseases such as severe organ failure; and pregnant or lactating women; subjects did not receive calcium channel blockers, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers, diuretics, and beta blockers before the study. We randomly recruited 260 healthy people from the medical examination center as the control group, including 132 men and 128 women, aged 35 to 80 years (mean, 54.29 ± 8.93) years according to the age and sex of EHT patients in a ratio of 1:1.

2.3. PTX3 genotype analysis

QIAamp DNA Blood Mini Kit (Cat No. 51104, Qiagen, German) was used to extract genomic DNA of monocytes from peripheral venous blood of all patients in accordance with the supplier’s instructions. Then, a polymerase chain reaction (PCR) was used to amplify the target fragment containing rs2614, rs111451363, and rs73158510 of PTX3. The amplified primer sequence was obtained using the Primer Blast tool in the National Center for Biotechnology Information (NCBI). The primer sequence of rs2614 site was as follows: forward primer: 5’-ACT TTT CGT CTC TCC AGC AA-3’ and reverse primer: 5’-CCA CAA GGA TGT GAG CCC TT-3’. The PCR reaction mixture contained 25 ng of genomic DNA, 2 μL of 10 × PCR buffer, 1.5 μL of 2.5 mmol/L dNTP, 10 μmol/L of forward and reverse primers, and 0.5 U Taq DNA polymerase. The PCR was performed in the following environment: 94°C, 5 minutes; (94°C, 30 seconds; 60°C, 30 seconds; 72°C, 1 minute) for 35 cycles; 72°C, 10 minutes. Products amplified by PCR were sequenced by Sangon, and 30% of samples were randomly selected for repeated verification, and the consistency rate of 2 sequencing results was 100%. According to the sequencing results and the PTX3 sequence alignment in NCBI, rs2614, rs111451363, and rs73158510 genotypes of PTX3 were determined.

2.4. Enzyme-linked immunosorbent assay (ELISA)

About 5 mL of fasting venous blood was collected from each participant and left at room temperature for 0.5 to 1 hours. After centrifugation, the upper plasma was retained and stored in a −80°C refrigerator for testing. Human Pentraxin 3 enzyme-linked immunosorbent assay Kit (ab214570, Abcam, Cambridge, UK) was used to calculate and measure plasma PTX3 levels in triplicate according to the standard curve method.

2.5. Quantitative real-time PCR

TRIzol (Gibco, USA) was used to extract total RNA from the plasma. Using the extracted RNA as a template, cDNA was synthesized using reverse transcription PCR. Then, the relative expression of hsa-miR-4766-5p was detected using cDNA as template and U6 as internal reference. The reaction system contains 2 × qPCR Mix 10 μL, Universal Adaptor primer 2 μL, forward/reverse primer 2 μL, 50 × Rox reference Dye 0.4 μL, cDNA 2 μL, the rest is ddH2O, and the total volume is 20 μL. The reaction conditions were as follows: Stage 1: 95°C, 10 minutes. Stage 2 50 cycles, 95°C, 10 seconds; 60°C, 20 seconds; 72°C, 10 seconds; and Stage 3: 95°C, 1 minute; 55°C, 30 seconds; 95°C, 30 seconds. The expression level of hsa-miR-4766-5p relative to U6 was expressed in $2^{-\Delta\Delta C_{t}}$ in triplicate.

2.6. Double luciferase reporting experiment

The T and C alleles containing the rs2614 locus of PTX3 gene were amplified from human genomic DNA as a template using PCR and inserted into a pGL3 vector (Promega Corporation, Madison, WI). Lipofectamine 2000 (Invitrogen) was used to co-transfect T and C alleles with hsa-miR-4766-5p mimic and hsa-miR-4766-5p inhibitor to HEK293 cells (Promega, Madison, WI). Luciferase activity was measured in triplicate for each group.

2.7. Statistical analysis

In this study, SPSS20.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. The $\chi^2$ test was used to assess whether the
selected population met the Hardy–Weinberg equilibrium. The binary logistic regression adjusted for factors such as age, gender, body mass index (BMI), smoking history, drinking history, diabetes history, and dyslipidemia history. The odds ratio (OR) and its 95% confidence interval (CI) were used to evaluate the correlation between the genotype and allele of the SNP locus of $PTX3$ and EHT risk. Multi-dimensional dimensionality reduction 3.0.2 software was used to analyze the effects of interaction between the SNP loci of $PTX3$ on EHT risk. Haploview 4.2 analyzed the linkage disequilibrium of rs2614, rs111451363, and rs73158510 loci. Pearson correlation analysis was used to assess the correlation between plasma $PTX3$ levels and hsa-miR-4766-5p levels. A $P < .05$ indicates a statistically significant difference.

3. Results

3.1. General characteristics of patients with EHT and control groups

The general clinical characteristics of 260 patients with EHT and 260 controls in this study are shown in Table 1. Results of the analysis showed that the difference in age, sex, smoking history, drinking history, and plasma HDL-C level was not significant in patients with EHT ($P > .05$). BMI, SBP, DBP, LDL-C, the proportion of patients with diabetes history, the proportion of patients with dyslipidemia history, and total cholesterol and triglyceride levels were significantly higher in patients with EHT than that in the control group ($P < .05$).

3.2. $PTX3$ gene 3’UTR SNP locus genotype and allele frequency comparison

In this study, genotype frequencies of rs2614, rs111451363, and rs73158510 loci of $PTX3$ in 260 subjects in control groups were enrolled in accordance with Hardy–Weinberg equilibrium ($P > .05$) (Table 2). After adjusting for factors such as age, sex, BMI, smoking history, drinking history, diabetes history, and dyslipidemia history, $PTX3$ rs2614 locus CT genotype and TT genotype carriers were significantly increased their risk of EHT by 1.74 times (95% CI: 1.08–2.79, $P = .03$) and 2.01 times (95% CI: 1.54–2.18, $P < .01$). No significant change was observed in the risk of EHT in the additive model, whereas the risk of EHT in the dominant model and the recessive model increased by 2.35 times (95% CI: 1.51–3.66, $P < .01$) and 1.91 times (95% CI: 1.47–2.07, $P < .01$). Carriers of the T allele at rs2614 locus of $PTX3$ gene were 2.76 times more likely to develop EHT than carriers of C allele (95% CI: 1.86–4.09, $P < .01$). No statistically significant difference was observed between different genotypes and allele frequencies of $PTX3$ gene rs111451363 and rs73158510 loci in patients with EHT and the control group ($P > .05$).

This indicates that SNP at rs2614 locus of $PTX3$ gene is significantly associated with the risk of EHT. Compared with C allele, T allele is a high risk factor for EHT. No correlation was observed between $PTX3$ gene rs111451363 locus and rs73158510 locus SNP and the risk of EHT.
3.3. Hierarchical analysis

We stratified the age, sex, BMI, smoking history, drinking history, diabetes history, and dyslipidemia history. Results showed that patients aged <60 years, ≥60 years, men, BMI <24 kg/m², ≥24 kg/m², with smoking history, no smoking history, drinking history, no drinking history, diabetes history, and dyslipidemia history, had a significantly increased risk of EHT in carriers of T allele (CT/TT) at rs2614 locus of PTX3 gene (P < .05). However, in women and people with diabetes history, no significant change was observed in the risk of EHT from carriers of T allele of PTX3 at rs2614 (CT/TT) (P > .05, Table 3). This shows that sex and diabetes history affect the correlation between SNP at rs2614 locus of PTX3 and EHT risk.

People without diabetes history, carriers of T allele (CT/TT) at rs111451363 locus of PTX3 have significantly increased the EHT risk (P < .05). However, in participants aged <60 years, ≥60 years, with BMI <24 kg/m², ≥24 kg/m², smoking history, no smoking history, drinking history, no drinking history, diabetes history, history of dyslipidemia, no significant difference was observed in the risk of EHT for T allele carrier (CT/TT) of PTX3 at rs111451363 (P > .05, Table 4). This shows that rs111451363 locus SNP of PTX3 was significantly associated with the EHT risk only in people without diabetes (P < .05).

In people aged ≥60 years and BMI of ≥24 kg/m², the carrier of PTX3 rs73158510 locus A allele (GA/AA) had significantly increased the EHT risk (P < .05). However, in participants aged <60 years, BMI <24 kg/m², with or without smoking history, drinking history, and diabetes history, dyslipidemia history, no significant change was observed in the EHT risk for rs73158510 locus of PTX3 (P > .05, Table 5). This shows that only in the population aged ≥60 years and BMI of ≥24 kg/m², the SNP at rs73158510 locus of PTX3 was significantly associated with the EHT risk (P < .05).

3.4. PTX3 SNP–SNP site interaction

We used a multi-dimensional dimensionality reduction method to analyze the correlation between the SNP–SNP site interactions at rs2614, rs73158510, and rs111451363 sites of PTX3 and the EHT risk. The results showed that rs2614, rs73158510, and rs111451363 interaction models were the best models for predicting EHT risk. The cross-validation consistency (CVC) was 10/10, and the accuracy was 58.93% (x² = 8.54, P = .01) (Table 6). The effect of rs2614, rs111451363, and rs73158510 loci on the EHT risk decreased in turn. The interaction between rs2614 and rs73158510 loci was stronger, followed by rs111451363 locus (Fig. 1A). People who also carry rs2614 TT genotype, rs73158510 GG genotype, and rs111451363 CC genotype are high risk factors for EHT (OR = 3.21, 95% CI: 2.05–4.22, P < .01). People carrying rs2614 CC genotype, rs73158510 GG genotype, and rs111451363 CC genotype are protective factors for EHT (OR = 0.78, 95% CI: 0.65–0.89, P = .02) (Fig. 1B).

Table 3

| Age | EHT (n=260) | Control (n=260) | OR (95% CI) | P |
|-----|------------|----------------|-------------|---|
| <60 | CC 136 (71.58%) 163 (84.02%) | 1.00 (Reference) | | |
|     | CT/TT 54 (28.42%) 31 (15.98%) | 2.09 (1.27–3.43) | <.01 | |
| ≥60 | CC 51 (72.86%) 60 (90.91%) | 1.00 (Reference) | | |
|     | CT/TT 19 (27.14%) 6 (9.09%) | 1.65 (1.12–2.09) | .01 | |
| Sex | Male | | | |
|     | CC 90 (70.87%) 116 (87.88%) | 1.00 (Reference) | | |
|     | Female | | | |
|     | CC 97 (72.93%) 107 (83.59%) | 1.00 (Reference) | | |
| BMI, kg/m² | <24 | | | |
|     | CC 67 (69.07%) 79 (86.81%) | 1.00 (Reference) | | |
|     | CT/TT 30 (30.93%) 12 (13.19%) | 1.33 (0.99–1.67) | .06 | |
| ≥24 | CC 120 (73.62%) 144 (85.21%) | 1.00 (Reference) | | |
|     | CT/TT 43 (26.38%) 25 (14.79%) | 1.39 (1.07–1.72) | .01 | |
| Smoking | | | | |
|     | Ever | | | |
|     | CC 49 (70.00%) 60 (90.91%) | 1.00 (Reference) | | |
|     | Never | | | |
|     | CC 138 (72.63%) 163 (84.02%) | 1.00 (Reference) | | |
| Drinking | | | | |
|     | Ever | | | |
|     | CC 49 (69.01%) 57 (91.94%) | 1.00 (Reference) | | |
|     | Never | | | |
|     | CC 138 (73.02%) 166 (83.84%) | 1.00 (Reference) | | |
| Diabetes | | | | |
|     | Ever | | | |
|     | CC 99 (69.23%) 45 (63.33%) | 1.00 (Reference) | | |
|     | Never | | | |
|     | CC 88 (75.21%) 178 (86.41%) | 1.00 (Reference) | | |
| Dyslipidemia | | | | |
|     | Ever | | | |
|     | CC 123 (73.65%) 68 (89.47%) | 1.00 (Reference) | | |
|     | Never | | | |
|     | CC 29 (24.79%) 28 (13.59%) | 1.54 (1.08–2.07) | .02 | |

BMI = body mass index, CI = confidence interval, EHT = essential hypertension, OR = odds ratio.

3.5. Haplotype analysis

Haploview 4.2 analyzed the linkage disequilibrium of rs2614, rs111451363, and rs73158510 loci. The results showed that rs2614, rs73158510 locus D was the highest, and rs111451363, rs73158510 locus D was the lowest (Fig. 2). Four haplotypes were formed at rs2614, rs111451363, and rs73158510 loci, of which the CCG haplotype was a protective factor of EHT (OR = 0.40, 95% CI: 0.28–0.57, P < .01), and TCG haplotype was a risk factor for EHT (OR = 2.35, 95% CI: 1.51–3.66, P < .01) (Table 7).

3.6. Analysis of plasma PTX3 and hsa-miR-4766-5p levels

We used enzyme-linked immunosorbent assay to detect plasma PTX3 levels in 260 patients with EHT and 260 control groups.
The results showed that plasma PTX3 levels in patients with EHT were significantly higher than those in the control group (P < .01, Fig. 3A). The receiver-operating curve analysis showed that the area under the curve for the diagnosis of EHT in plasma PTX3 levels was 0.62 (95% CI: 0.57–0.66, P < .01, Fig. 3B). Quantitative real-time PCR detected plasma hsa-miR-4766-5p levels. Results showed that plasma hsa-miR-4766-5p levels in patients with EHT were significantly lower than those in the control group (P < .01, Fig. 3C). The analysis showed that the area under the curve for the diagnosis of EHT by plasma hsa-miR-4766-5p level was 0.88 (95% CI: 0.85–0.91, P < .01, Fig. 3D). Further analysis of the correlation between plasma PTX3 levels and hsa-miR-4766-5p levels, results showed that plasma PTX3 levels and hsa-miR-4766-5p levels in patients with EHT and controls were significantly negatively correlated (r = –0.87, –0.85, P < .01, P < .01, Fig. 3E, F).
3.7. C allele of rs2614 at PTX3 gene is the target gene of hsa-miR-4766-5p

Results of our bioinformatics analysis showed that the C allele of the rs2614 locus of PTX3, instead of T allele, had a target binding site for hsa-miR-4766-5p (Fig. 4A). To further analyze whether hsa-miR-4766-5p binds to the site predicted by C allele of rs2614 locus of PTX3, a double luciferase reporter assay was used. Results showed that t PTX3 rs2614 locus C allele and hsa-miR-4766-5p mimic co-transfected significantly reduced fluorescence activity; however, The fluorescence activity were increased after co-transfection of the PTX3 rs2614 locus C allele and hsa-miR-4766-5p inhibitor (Fig. 4B). This indicates that C allele at rs2614 locus of PTX3 gene is the target gene of hsa-miR-4766-5p, not T allele.

| Model                        | Accuracy | CVC | \(\chi^2\) | \(P\) |
|------------------------------|----------|-----|------------|-------|
| rs2614                       | 56.92%   | 8/10| 3.52       | .09   |
| rs2614, rs73158510           | 57.84%   | 7/10| 4.12       | .06   |
| rs2614, rs73158510, rs111451363| 58.93%  | 10/10| 8.54       | .01   |

\(\text{CVC} = \text{cross-validation consistency, EHT = essential hypertension, MDR = multifactor dimensionality reduction.}\)

Table 6

Figure 1. MDR analysis of rs2614, rs73158510, and rs111451363 site interactions. (A) Cyclic and dendrogram analysis of SNP–SNP interactions. Data below the SNP site represent the effect on the risk of EHT. The data on the connection line represent the magnitude of the SNP–SNP interaction. The larger the value, the stronger the interaction. (B) Graphical Model, “a” indicates the model with the lowest risk of EHT, and “b” indicates the model with the highest risk of EHT. EHT = essential hypertension.

Figure 2. Haploview 4.2 Analysis of haplotypes of PTX3 SNP loci.
4. Discussion

In this study, a case–control study was used to analyze the correlation between PTX3 rs2614, rs111451363, and rs73158510 loci and the EHT risk. Results showed that carriers of T allele at rs2614 locus of PTX3 were 2.76 times more likely to have EHT than carriers of C allele (95% CI: 1.86–4.09, \(P < .01\)), and sex and diabetes history were interfering factors. We found that CCG haplotype is a protective factor for EHT, and TCG haplotype is a risk factor for EHT. The plasma PTX3 level of patients with EHT was significantly higher than that of the control group, and the plasma hsa-miR-4766-5p level of patients with EHT was significantly lower than that of the control group (\(P < .01\)). Further research showed that plasma PTX3 levels and hsa-miR-4766-5p levels in patients with EHT and controls were significantly negatively correlated. Bioinformatics combined with the analysis of results in the double luciferase report confirmed that C allele at rs2614 locus of PTX3 instead of T allele was the target gene of hsa-miR-4766-5p.

Hypertension is one of the most common diseases that endanger human health and is one of the major risk factors for atherosclerosis. Long-term elevated blood pressure causes and promotes the formation and development of atherosclerosis. Immune and inflammation run through the whole process of the occurrence and development of atherosclerosis, of which, inflammation may be a bridge connecting hypertension and atherosclerosis,[17,18] and inflammation is an important participant on the mechanism of hypertension in atherosclerosis.

PTX3 consists of three exons and encodes a total of 381 amino acids. PTX3 plays an important role in innate immunity,[19] inflammatory response,[20] vascular integrity, fertility, pregnancy, and central nervous system. The normal function of PTX3 in innate immunity and inflammation is to selectively strengthen the immune response to some pathogens, while controlling the potential autoimmune response.

PTX3 is closely related to the occurrence and development of atherosclerosis and can play a cardiovascular protective role by balancing immune inflammation.[21] One of the main biologically active substances of oxLDL, lysophosphatidic acid, stimulates human endothelial cells to cause increased PTX3 secretion.[22] Studies have shown that patients with unstable angina pectoris,[23] acute myocardial infarction,[24] and chronic heart failure[20] have significantly increased blood PTX3 levels. Therefore, PTX3 is speculated as an important indicator of vascular inflammation and cardiovascular system damage. Few studies investigated the correlation between PTX3 and EHT. In this study, we found that PTX3 levels were significantly elevated in patients with EHT, and we speculate that this may be due to increased levels of PTX3 secreted by macrophages and vascular endothelial cells; however, elevated PTX3 levels can better reflect the inflammatory state of the vascular bed, representing EHT subclinical AS and early kidney damage.

| Haplotypente | EHT (n = 260) | Control (n = 260) | OR (95% CI) | \(P\) |
|--------------|--------------|-----------------|------------|------|
| CCG          | 108 (41.54%) | 166 (63.85%)    | 0.40 (0.28–0.57) | < .01 |
| CCA          | 52 (20.00%)  | 38 (14.62%)     | 1.19 (0.95–1.45) | .13  |
| TCG          | 73 (28.08%)  | 37 (14.23%)     | 2.35 (1.51–3.66) | < .01 |
| CTG          | 27 (10.38%)  | 19 (7.31%)      | 1.19 (0.87–1.51) | .28  |

\(CI = \) confidence interval, EHT = essential hypertension, OR = odds ratio.

\*rs2614, rs111451363, rs73158510.

**Figure 3.** Analysis of plasma PTX3 and hsa-miR-4766-5p levels. (A) ELISA detects plasma PTX3 levels. (B) ROC analysis of the diagnostic value of plasma PTX3 levels for EHT. (C) qRT-PCR detection of plasma hsa-miR-4766-5p levels. (D) ROC analysis of plasma hsa-miR-4766-5p levels in the diagnosis of EHT. (E) Correlation between plasma PTX3 and hsa-miR-4766-5p levels in patients with EHT. (F) Correlation between plasma PTX3 and hsa-miR-4766-5p levels in the control group. EHT = essential hypertension.
In this study, SNP at rs2614 locus of PTX3 is found to be associated with the EHT risk. We know that the risk of hypertension may be different in people of different genetic backgrounds. Therefore, a stratified analysis of age, sex, BMI, smoking history, drinking history, diabetes history, and dyslipidemia history was performed. Results showed that sex and diabetes history affected PTX3 rs2614 loci SNP and the EHT risk. This shows that the correlation between SNP at rs2614 locus of PTX3 and the EHT risk is related to sex and dyslipidemia history. However, it is interesting to note that we did not find any correlation between PTX3 rs111451363 and rs73158510 loci SNP and the EHT risk. However, in people without diabetes history, the SNP at rs111451363 at PTX3 was significantly associated with the EHT risk ($P < .05$). Only in the population aged >60 years and BMI of ≥24 kg/m$^2$, the SNP at rs73158510 locus of PTX3 was significantly associated with the EHT risk ($P < .05$). We speculate that it may be related to the small sample size in this study, and whether there is correlation after enlarging the sample size needs further verification.

MicroRNA is a type of endogenous noncoding RNA widely distributed in the body, with a length of about 20 nucleotides (PMID: 27826912). Studies have shown that microRNAs play an important role in the occurrence and development of essential hypertension. For example, miRNAs are related to the excessive activation of the renin-angiotensin-aldosterone system, and affect blood pressure indirectly or directly through a variety of ways (PMID: 24799609). Liu et al’s (PMID: 30049682) study showed that MicroRNA-214-3p in the kidney contributes to the development of hypertension. Liu et al (PMID: 30483753) research showed that miR-140-5p aggravated hypertension and oxidative stress in atherosclerotic mice by targeting Nrf2 and Sirt2.

To explore the possible mechanism for this correlation, the correlation between PTX3 levels and hsa-miR-4766-5p levels was analyzed in the plasma of patients with EHT and control groups. Results showed a significant negative correlation between plasma PTX3 levels and hsa-miR-4766-5p levels in patients with EHT and control groups. We speculated that hsa-miR-4766-5p may have a negative regulatory effect on PTX3 expression. Therefore, we used double luciferase reporting experiments to confirm that PTX3 rs2614 locus C allele rather than T allele is the target gene of hsa-miR-4766-5p. However, the regulation of PTX3 by other miRNAs has not been affected by SNPs at rs111451363 and rs73158510.

In addition, taking the allele frequency of rs2614 as a reference, the minimum sample size needed in this study was calculated. The results showed that the minimum sample size required for EHT patients and the control group was 148 cases; 148 cases showed that the results of this study have a certain degree of credibility.

This study provides new ideas for the prevention and treatment of EHT. The difference in the risk of EHT among populations of different genetic backgrounds may be related to SNP of key genes. Simultaneously, it reminds us that we cannot just focus on the role of miRNAs in regulating the expression of key genes. The reasons behind them are also worthy of further discussion, such as SNP at the target.

In addition, the allele frequency of rs2614 locus was used as a reference to calculate the minimum sample size required in this study. The results showed that the minimum sample size required for EHT patients and the control group were 148 cases, respectively. One hundred forty-eight cases indicate that the results of this study have a certain degree of credibility.

There are some limitations in this study that require further study. First, we need to expand the sample size for research to further analyze whether SNPs at rs111451363 and rs73158510 are related to the EHT risk. Second, we have not yet verified the role of hsa-miR-4766-5p in regulating PTX3 expression in vitro models. In addition, we have not yet verified the effect of PTX3 on EHT in an in vivo model, and whether the expression level of PTX3 in individuals with different alleles at rs2614 locus is related to the difference in the modulation of hsa-miR-4766-5p.

To summarize, the SNP of rs2614 locus of PTX3 is significantly related to the EHT risk, which may be related to the difference in the targeted binding of different alleles of rs2614 locus to hsa-miR-47665p. The specific mechanism needs further research.

**Author contributions**

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