Genetic associations between miR-200bT>C and miR-495A>C polymorphisms and hypertension susceptibility

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Abstract. Although microRNA (miRNA)-mediated functions and gene expression regulation are involved in the susceptibility to vascular diseases, the potential effect of miRNA polymorphisms on the susceptibility of patients to hypertension (HTN) remains to be sufficiently elucidated. Therefore, the present study aimed to identify the potential association between miRNA (miR)-200bT>C (rs7549819) and miR-495A>C (rs2281611) polymorphisms, which may be implicated in stroke and vascular pathogenesis, and the susceptibility to HTN and relevant risk factors in a Korean cohort recruited from Jeju National University Hospital (Jeju, South Korea). Using an analysis of PCR-restriction fragment length polymorphism, genotype analysis was conducted to assess the frequency of miR-200bT>C and miR-495A>C gene polymorphisms in the HTN group (n=232) and the non-HTN healthy control group (n=247). The results showed significant differences in the genotype distributions of the miR-495A>C polymorphism between the HTN and control groups, specifically with the CC genotype and C allele. However, neither the miR-200bT>C nor the dominant and recessive models were found to be distributed differently between the two groups. Following analysis of the genotype combination of the single nucleotide polymorphisms, the TC/CC and CC/CC combined genotypes of the miR-200bT>C and miR-495A>C polymorphisms were observed to be associated with susceptibility to HTN. The haplotype results demonstrated that the allele combination frequency of haplotype C-A was significantly different between the two groups. The stratified analysis revealed that the miR-200b and miR-495 polymorphisms are associated with the risk of HTN, exhibiting differences in the levels of body-mass index (<28.12 kg/m^2), fasting blood glucose (<106.26 mg/dl), high-density lipoprotein cholesterol (<44.29 mg/dl) and systolic blood pressure (≥132.67 mmHg). Data from the present study suggested that the variant of miR-495A>C polymorphism and allelic combinations (haplotype C-A of miR-200bT>C/miR-495A>C) can increase hypertension susceptibility among a Korean population.

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Introduction

Hypertension (HTN) is a chronic illness with uncertain etiology. It is a common cardiovascular risk factor that contributes to the likelihood of cardiovascular events (1,2). Severe myocardial injury, heart failure, chronic kidney disease, peripheral vascular disease, stroke and derived complications affecting organ systems, such as the brain and eyes, are known to be associated with HTN (3-5). According to the latest report in December 2022 conducted by the Korea Centers for Disease Control and Prevention, HTN affects 33.2% of the Korean population aged >30 years and 62.3% of the population aged >65 years (6). Similar to other vascular conditions, HTN has been characterized by the aberrant expression of microRNAs (miRNAs/miRs), which can induce translational repression or mRNA degradation (2,4). Since the pathophysiology of HTN constitutes an intricate combination of environmental and genetic factors, our understanding of the molecular mechanism underlying this condition remains incomplete (4).

miRNAs are a conserved class of small, non-coding RNAs that mainly function to block the translation of or accelerating the degradation of mRNAs by binding to their 3'-untranslated regions (3'-UTRs) (7-9). Through this function, they have been documented to, in turn, regulate endothelial function, the renin-angiotensin-aldosterone system and the pathogenesis of HTN (10). Investigations into these miRNAs have the potential to contribute to our understanding of the pathological mechanisms underlying HTN and may lead to the development of therapeutic medicine against this condition (4). A previous study by Yin *et al* (5) analyzed miR-128 expression in the peripheral blood of patients with HTN in China; they found that upregulation of this miRNA was associated with the progression of myocardial injury in the HTN group, particularly stage III/IV HTN. Other previous studies have shown the expression of members of the miR-29 family to be upregulated in patients with HTN, whilst also showing a positive association with their blood pressure (2). Zhang *et al* (10) previously suggested that miR-122 bound to the 3'-UTR of cationic amino

acid transporter 1 mRNA may lead to the decreased metabolism of L-arginine and nitric oxide in vascular endothelial cells. This can eventually lead to the dysfunction of vascular endothelial cells, which have been reported to be a significant cause of HTN (10). In miRNA biology, although a single miRNA can target multiple genes, it can also be targeted by other genes. Owing to this feature, miRNAs can potentially serve an important role in a regulatory network controlling the pathological mechanisms of various cardiovascular diseases, such as HTN (4).

Previous studies have suggested that miRNA polymorphisms can contribute to a genetic predisposition to HTN (1,2,5,11). Members of the miR-200 family, including miR-200b, have been reported to serve an important role in processes associated with hypoxia, such as angiogenic responses in microvascular endothelial cells and the apoptosis of cardiomyocytes (12). They have also been found to have a significant relationship with tumorigenesis, cancer metastasis and recurrence (13). miR-200b expression has been shown to be increased in renal tissues of patients with hypertensive nephrosclerosis when accompanied by expression in heart ventricles with salt-sensitive hypertension (11). In addition, the dynamic expression of miRNAs, a genetic regulatory mechanism for platelet formation and activation, has been reported to be linked with the pathogenesis of thrombotic disorders (14). A prior study examining miRNA expression in platelets reported that the expression of miR-200b and miR-495 may be significantly downregulated during the maturation of megakaryocytes into platelets (15). These miRNA shave also been shown to reduce the expression of platelet-specific functional proteins, protein kinase cAMP-dependent type II regulatory subunit β (PRKAR2B) and Kelch-like family member 5 (KLHL5) (16). Therefore, the target genes of miR-200b and miR-495 may modulate platelet functions during the activation, aggregation and proinflammatory reaction of platelets (14).

Polymorphisms of miR-200bT>C (rs7549819) in chromosome 1 and miR-495A>C (rs2281611) in chromosome 14 were previously found in their corresponding promoter regions (14,17). Their genetic variant allele frequencies in the global population from the Allele Frequency Aggregator dataset were documented to be 0.099 for miR-200bT>C and 0.236 for miR-495A>C (17). In a study by Kim *et al* (14), susceptibility to ischemic stroke and post-stroke mortality was investigated among cohorts with various miRNA genotypes. The study did not find a significant difference in the distribution of miRNA single nucleotide polymorphisms (SNPs; miR-200bT>C and miR-495A>C) between the patient and control groups, but the miR-200b CC genotype was less frequently found in patients with large-artery atherosclerotic stroke (14). Furthermore, Qin *et al* (18) previously suggested that the miR-200bT>C and miR-495A>C variants were significantly associated with genes and pathways that can regulate ischemic stroke pathogenesis in a Chinese population. In addition, their evidence has also supported the notion that alterations in the miR-200b and miR-495 genetic structure were associated with the promotion of neovascularization in ischemia and myocardial infarction mouse models (18).

However, although miR-200b and miR-495 are likely to be involved in several diseases, including lung, prostate, breast, colon and endometrial cancer, to the best of our knowledge, no studies on the association of the polymorphisms of miR-200bT>C and miR-495A>C with HTN. Therefore, the present study aimed to test the hypothesis of the potential association between miR-200bT>C and/or miR-495A>C polymorphisms and susceptibility to HTN in a Korean cohort.

Materials and methods

Study population. For the present study, 232 patients with HTN, including 190 male patients and 42 female patients (mean ± SD age, 47.35±8.23 years; age range, 31-67 years), and 247 control individuals, including 132 men and 115 women (mean ± SD age, 48.93±9.98 years; age range, 32-80 years), were enrolled. The patients with HTN, defined as systolic pressure >140 mmHg or diastolic pressure >90 mmHg, were recruited from Jeju National University Hospital (Jeju, South Korea) between January 2020 and December 2020. Healthy control individuals had normal blood pressure and were not on medication, and were enrolled during the same period. Patients who were diagnosed with other chronic diseases were excluded from the current study. The blood pressure values of all participants were measured in a sitting position after resting for ≥10 min. HTN would be diagnosed if three repeated measurements of the systolic blood pressure (SBP) were >140 mmHg and the diastolic blood pressure (DBP) were >90 mmHg. Risk factors for hypertension were also measured. Body mass index (BMI) was calculated from measurements of height and weight. Waist circumference (WC) was measured using a non-stretchable fiber measuring tape. For biochemical measurements, the levels of plasma fasting blood glucose (FBG) (cat. no. GLU CN R1 991-18592, cat. no. GLU CN R2 997-18692; FUJIFILM Wako Pure Chemical Corp.), triglycerides (cat. no. TG CN R1 993-33192, cat. no. TG CN R2 999-33292; FUJIFILM Wako Pure Chemical Corp.) and high-density lipoprotein-cholesterol (HDL-C) (cat. no. ML HDL-C S R1 55379, cat. no. ML HDL-C S R2 55380; Minaris Medical Co., Ltd.) were measured using commercially available enzymatic colorimetric tests in an automated analyzer (TBA 200FR NEO; Canon Medical Systems Corporation). Participants in the control group were randomly selected to be subjected to a medical examination, which excluded individuals with a history of chest pain, diabetes, and hypertension. All enrolled subjects provided written informed consent to participate in the present study, and ethical approval was received from the Institutional Review Board of Jeju National University Hospital (Republic of Korea; approval no. JEJUNUH 2020-07-005). The biospecimen and data used in the present study were provided by the Biobank of Jeju National University Hospital, a member of the South Korea Biobank Network supported by the Ministry of Health and Welfare.

Genotyping. Genomic DNA was extracted from white blood cells in 3-ml blood samples taken twice 1 month after the date of HTN diagnosis using a G-DEX blood extraction kit (iNtRON Biotechnology, Inc.). Genotype analysis of miR-200bT>C (rs7549819) and miR-495A>C (rs2281611) polymorphisms was conducted using PCR and the restriction fragment length polymorphism technique, as described previously (14). Briefly, genomic DNA was amplified using PCR

Table I. Details of the SNPs (miR-200bT>C and miR-495A>C) included in the present study.

SNP	Primer sequence[a]	PCR annealing temperature (product length, bp)	Genotype (length, bp)	Restriction enzyme
miR-200bT>C	Forward primer 5'-CCTGAACCTGGCAGTGG-3'; Reverse primer 5'-CAGTGCTTCAGGAACACAATTT-3'	60˚C (209)	TT (209); TC (209, 179, 30); CC (179, 30)	*Aci*I
miR-495A>C	Forward primer 5'-GCATCAGGTAAGTTGGGTCA-3'; Reverse primer 5'-TTATCCGTGATGACTGTCCG-3'	60˚C (94)	AA (94); AC (94, 74, 20); CC (74, 20)	*Hinc*II

[a]Underline indicates the nucleotide of miR-495A>C SNP mismatch sequence. miR, microRNA; SNP, single nucleotide polymorphism.

Table II. Baseline characteristics of the study cohort[a].

Clinicopathological characteristic	Control (n=247)	Hypertension (n=232)	P-value
Age, year	48.93±9.98	47.35±8.23	0.09
Body mass index, kg/m^2	23.94±3.45	26.14±3.42	<0.0001
Waist circumference, cm	81.33±8.65	87.23±9.22	<0.0001
Systolic blood pressure, mmHg	118.51±11.24	138.43±13.44	<0.0001
Diastolic blood pressure, mmHg	71.36±8.63	88.55±8.85	<0.0001
Fasting blood glucose, mg/dl	88.18±8.91	95.48±14.18	<0.0001
Triglyceride, mg/dl	92.15±57.38	133.52±96.00	<0.0001
High-density lipoprotein-cholesterol, mg/dl	57.45±14.25	52.21±11.25	<0.0001

[a]Results are presented as the mean ± standard deviation.

premix kits (cat. no. STD02-P096; SolGent Co., Ltd.). PCR conditions were set at 94˚C for 5 min initial denaturation, followed by 32 cycles at 94˚C for 30 sec denaturation, 60˚C for 30 sec annealing and 72˚C for 1 min extension, and a final step at 72˚C for 5 min. After restriction digestions were performed at 37˚C for 17 h with *Aci*I and *Hinc*II restriction enzymes (New England BioLabs, Inc.), the samples were finally subjected to electrophoresis for 40 min at 150 V on 3 or 4 % agarose gel containing ethidium bromide. The primer sequences, PCR conditions, restriction enzymes and genotype fragments used are summarized in Table I. The following reference sequence shows the amplification region for analyzing the miR-200b polymorphism (underlined bases indicate the positions of the primers, where * is the *Aci*I cutting site): 5'-CTGAACCTG GCAGTGGGAGGCCGGCCCGTC*GCCGGGTGGGCT GGGAACTGTGTTTGGCCCCAGCTGCAAGGGACGAGT GCCGGGCACCTGCTGCCCTCCCCACGTGACCTGGCA GCCAGGAATGGAGCTGAAATTCAACTCTGCTCTGAA CGGGAAGTCTTAGTTTCCTTAGCTCAGAAAACAGGT GAAATTGTGTTCCTGAAGCACTG-3'.

The following sequence shows the amplification region for analyzing the miR-495 polymorphism (underlined text indicate the positions of the primers, where * is the *Hinc*II cutting site): 5'-GCATCAGGTAAGTTGGGTC*ACCCAGGGGAG GTGGGGGACATGCTTCGAAGGGCTGGGCTGACACTG AGCAGAGGCGGACAGTCATCACGGATAA-3'.

Statistical analysis. All statistical analyses were performed using GraphPad Prism version 4.0 (GraphPad Software Inc.; Dotmatics, Inc.) and MedCalc version 12.7.1.0 (MedCalc Software Ltd.). To compare the clinical characteristics of the study groups, the unpaired samples t-test or Mann-Whitney test were used for categorical variable and continuous variable analyses, respectively. The adjusted odds ratios and 95% confidence intervals for association with the miR-200bT>C (rs7549819) and miR-495A>C (rs2281611) polymorphisms in terms hypertension risk were calculated using multivariate logistic regression following adjustment for age and sex. P<0.05 was considered to indicate a statistically significant difference.

Results

Demographic information. The demographics of the HTN patient group and the non-HTN group are summarized in Table II. Compared with those in the control group, the HTN group had significantly higher BMI, WC, SBP, DBP and FBG (all P<0.001). By contrast, the HDL-C levels in the HTN

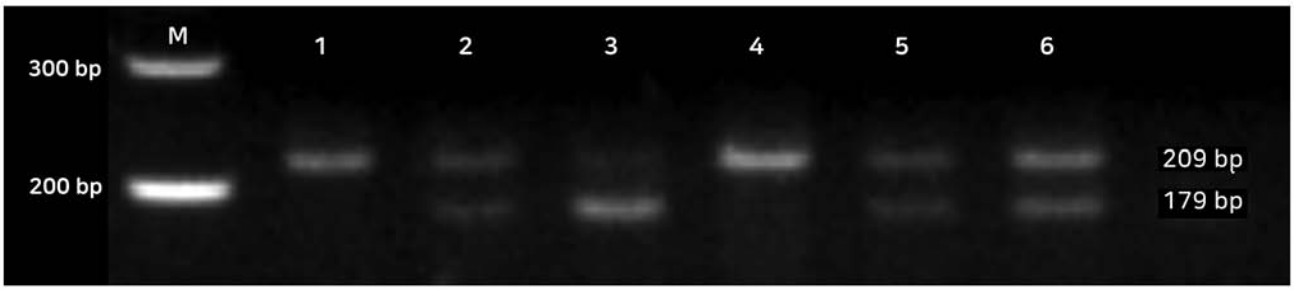

Figure 1. Restriction fragment length polymorphism results showing the restriction enzyme *Aci*I digestion pattern of miR-200b rs7549819. M, 100-bp size marker; lanes 1 and 4, wild-type homozygous alleles (TT); lane 3, mutant-type homozygous allele (CC); lanes 2, 5 and 6, heterozygous alleles (TC). miR, microRNA.

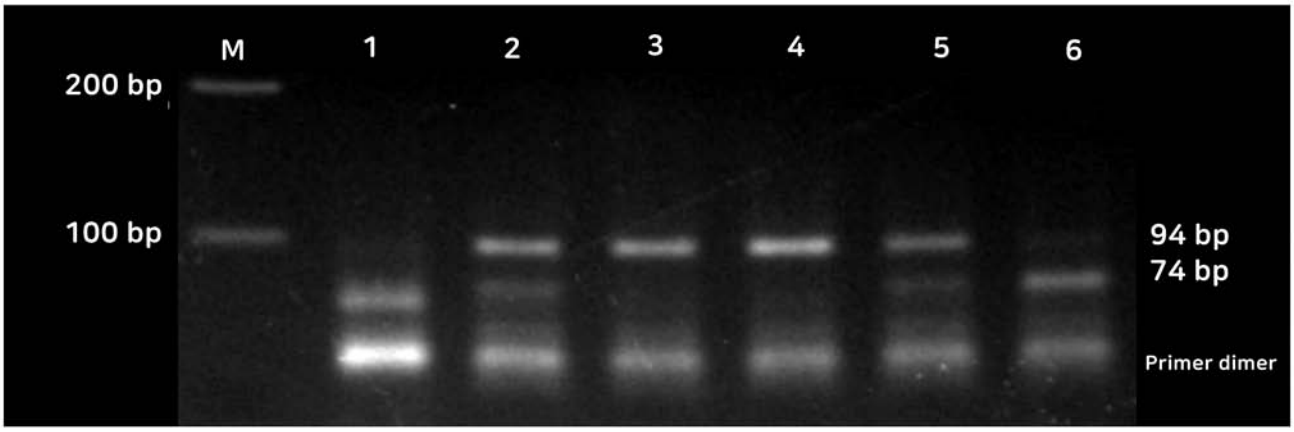

Figure 2. Restriction fragment length polymorphism results showing the restriction enzyme *Hinc*II digestion pattern of miR-495 rs2281611. M, 100-bp size marker; lanes 3 and 4, wild-type homozygous alleles (AA); lanes 1 and 6, mutant-type homozygous alleles (CC); lanes 2 and 5, heterozygous alleles (AC). miR, microRNA.

group were significantly lower compared with those in the control group (P<0.001). However, these two groups did not significantly differ in terms of age.

Detection of miR-200bT>C and miR-495A>C polymorphisms. For miR-200bT>C gene polymorphism, the genotypes were assessed as follow: A single 209 bp fragment for the wild-type homozygous alleles (TT genotype), two fragments of 179 and 30 bp for the mutated type (CC genotype), and three fragments of 209, 179 and 30 bp for the heterozygous alleles (TC genotype) (Fig. 1). The 30 bp fragment is not shown in Fig. 1. For miR-495A>C polymorphism, homozygosity of the common allele (AA genotype) revealed itself as a 94 bp band, while the homozygosity of the variant allele (CC genotype) was represented by 74 and 20 bp bands. The heterozygous alleles (AC genotype) were revealed by 94, 74 and 20 bp (Fig. 2). The 20 bp fragment is not shown in Fig. 2. The lowest bands in lanes 1-6 are primer dimers.

Association analysis. Table III presents the comparison of genotype and allele frequencies with regards to the miR-200bT>C and miR-495A>C polymorphisms between the HTN and control groups. No significant difference in the incidence in miR-200bT>C polymorphism could be observed between the two groups. Specifically, incidence in the miR-200b TC and CC genotypes were not significantly different, nor were the dominant and recessive models. In addition, no significant association could be detected between the HTN and control cohorts in terms of the allelic distribution of this miR-200bT>C polymorphism. By contrast, the miR-495 CC genotype (P<0.05) and C allele (P<0.01) were found to be significantly associated with the risk of HTN. There were no significant differences regarding the miR-495 AC genotype or between the dominant and recessive models.

Combined genotype analysis. To investigate the association between genotype combination and the risk of HTN, the combined genotypes of the miR-200bT>C and miR-495A>C polymorphisms in the HTN and control groups were then analyzed. According to the data shown Table IV, the TC miR-200bT>C/CC miR-495A>C (P<0.05) and CC miR-200bT>C/CC miR-495A>C (P<0.05) combined genotypes were found to be associated with a genetic susceptibility to HTN.

To assess the combined effect of the miR-200bT>C and miR-495A>C polymorphisms, allele combination analysis was subsequently conducted (Table V). The results demonstrated that the C-A allele combination frequency of miR-200bT>C and miR-495A>C was significantly higher in the HTN group compared with that in the control group (P<0.05).

According to a stratified analysis divided by the median values of the risk factors of the HTN group

Table III. Comparison of genotype and allele frequencies for the SNPs between the HTN and control cohorts.

A, miR-200b T>C

Genotype	Control, n (%)	HTN, n (%)	Odds ratio (95% CI)[a]	P-value
TT	96 (38.9)	81 (34.9)	1.000 (reference)	
TC	108 (43.7)	114 (49.1)	1.291 (0.850-1.960)	0.231
CC	43 (17.4)	37 (15.9)	1.002 (0.575-1.748)	0.994
Dominant (TT vs. TC+CC)			1.209 (0.817-1.788)	0.343
Recessive (TT+TC vs. CC)			0.870 (0.525-1.442)	0.589
T allele	300 (60.7)	276 (59.5)	1.000 (reference)	
C allele	194 (39.3)	188 (40.5)	1.053 (0.813-1.364)	0.694

B, miR-495A>C

Genotype	Control, n (%)	HTN, n (%)	Odds ratio (95% CI)[a]	P-value
AA	99 (40.1)	71 (30.6)	1.000 (reference)	
AC	110 (44.5)	106 (45.7)	1.258 (0.814-1.946)	0.301
CC	38 (15.4)	55 (23.7)	1.789 (1.033-3.098)	0.038
Dominant (AA vs. AC + CC)			1.413 (0.945-2.111)	0.092
Recessive (AA + AC vs. CC)			1.606 (0.992-2.599)	0.054
A allele	308 (62.3)	248 (53.4)	1.000 (reference)	
C allele	186 (37.7)	216 (46.6)	1.442 (1.115-1.866)	0.005

[a]Adjusted by age and sex of participants. CI, confidence interval; HTN, hypertension; miR, microRNA.

Table IV. Combined genotype analysis for SNPs in the HTN and control cohorts.

Genotype combination					
SNP 1 miR-200bT>C	SNP 2 miR-495A>C	Control, n (%)	HTN, n (%)	Odds ratio (95% CI)[a]	P-value
TT	AA	37 (15.0)	21 (9.1)	1.000 (reference)	
	AC	39 (15.8)	38 (16.4)	1.701 (0.804-3.600)	0.165
	CC	20 (8.1)	22 (9.5)	1.960 (0.808-4.756)	0.137
TC	AA	40 (16.2)	37 (15.9)	1.838 (0.856-3.946)	0.119
	AC	54 (21.9)	53 (22.8)	1.791 (0.865-3.709)	0.116
	CC	14 (5.7)	24 (10.3)	2.772 (1.091-7.043)	0.032[a]
CC	AA	22 (8.9)	13 (5.6)	1.139 (0.395-3.290)	0.810
	AC	17 (6.9)	15 (6.5)	1.504 (0.579-3.904)	0.402
	CC	4 (1.6)	9 (3.9)	4.692 (1.067-20.630)	0.041[a]

[a]Adjusted for age and sex of participants. CI, confidence interval; HTN, hypertension; miR, microRNA; SNP, single nucleotide polymorphism.

(Table VI), the recessive model (TT vs. TC+CC) of the miR-200b rs7549819 polymorphism was associated with an increased risk in the BMI (<28.12 kg/m^2; P<0.05) and FBG (<106.26 mg/dl; P<0.05) among the possible risk variables tested. Furthermore, the miR-495 AC+CC vs. AA of the rs2281611 recessive model was associated with increased HTN risk in the HDL-C (<44.29 mg/dl; P<0.05) and SBP (≥132.67 mmHg; P<0.05).

Discussion

Previous studies have reported that miRNAs in the human genome act as unknown multifactorial factors in the development of various diseases, such as ischemic stroke and thrombotic disorders, since they work as genetic modulators affecting target gene expression (4,14). This is due to miRNAs having been found to be associated with several physiological

Table V. Comparison of allele combination between the HTN and control cohorts.

Haplotype (miR-200bT>C/ miR-495A>C)	Overall(Control + HTN)	Control (n=548)	HTN (n=464)	Odds ratio (95% CI)	P-value
T-C	0.3274	0.3508	0.3014	1.000 (reference)	
T-A	0.2739	0.2565	0.2934	1.331 (0.958-1.850)	0.088
C-C	0.2530	0.2727	0.2330	0.994 (0.710-1.393)	0.974
C-A	0.1458	0.1200	0.1721	1.669 (1.115-2.498)	0.012

CI, confidence interval; HTN, hypertension; miR, microRNA.

Table VI. Stratified analysis of miR-200bT>C and miR-495A>C polymorphisms in the HTN group according to the HTN risk factors.

	miR-200b TC+CC		miR-495 AC+CC	
Variable	Odds ratio[a] (95% CI)	P-value	Odds ratio[a] (95% CI)	P-value
Body mass index, kg/m^2				
<28.12	1.627 (1.009-2.623)	0.046	1.289 (0.807-2.057)	0.288
≥28.12	0.864 (0.528-1.416)	0.563	1.418 (0.829-2.423)	0.202
Waist circumference, cm				
<93.03	1.326 (0.833-2.113)	0.235	1.452 (0.904-2.331)	0.123
≥93.03	1.077 (0.652-1.777)	0.773	1.211 (0.712-2.059)	0.480
Systolic blood pressure, mmHg				
<132.67	1.243 (0.765-2.019)	0.380	0.974 (0.599-1.583)	0.915
≥132.67	1.181 (0.735-1.896)	0.492	1.939 (1.156-3.252)	0.012
Diastolic blood pressure, mmHg				
<84.21	1.087 (0.648-1.824)	0.752	1.235 (0.725-2.102)	0.437
≥84.21	1.286 (0.815-2.028)	0.280	1.449 (0.904-2.323)	0.123
Fasting blood glucose, mg/dl				
<106.26	1.595 (1.002-2.540)	0.049	1.518 (0.949-2.430)	0.082
≥106.26	0.829 (0.499-1.375)	0.467	1.156 (0.679-1.966)	0.594
Triglyceride, mg/dl				
<216.26	1.358 (0.873-2.114)	0.175	1.332 (0.853-2.080)	0.207
≥216.26	0.990 (0.575-1.704)	0.971	1.390 (0.771-2.508)	0.274
High-density lipoprotein-cholesterol, mg/dl				
<44.29	1.015 (0.637-1.617)	0.950	1.855 (1.101-3.126)	0.020
≥44.29	1.549 (0.929-2.581)	0.093	0.937 (0.577-1.523)	0.793

[a]Adjusted for age and sex of participants. HTN, hypertension; miR, microRNA.

and pathological processes, such as oncogenesis and cardiovascular diseases, including coronary artery disease, stroke, acute myocardial infarction and heart failure (19). In particular, circulating miRNAs targeting other miRNAs as intercellular signaling mediators, for instance, circulating levels of miR-181a and miR-122 were higher in hypertensive individuals than in controls, have been proposed to serve a role in HTN according to tissue-based studies (1,4,10). According to results from the present study, miRNA polymorphisms miR-200bT>C and miR-495A>C are likely to be associated with the development of HTN in a cohort of Korean patients. Located in the promoters of the respective miRNAs, these miR-200b and miR-495 SNPs can influence their expression levels, which can in turn affect the expression levels of their target genes (20).

The results of the present study suggested an association of the miR-495 CC genotype and C allele with an increased risk of HTN. However, the risk of HTN was not associated with the miR-200b TC and CC genotypes, dominant and recessive models or the miR-200b variant T and C allele. miR-495 has been previously shown to be associated with susceptibility to blood-related diseases, such as pre-eclampsia (21), ischemic

stroke and post-stroke prognosis (14), by inhibiting cell proliferation, migration, invasion and angiogenesis. Hu *et al* (22) previously reported that miR-495 leads to the pathogenesis of type 2 diabetes since it could increase the blood glucose content and insulin resistance by a targeted negative regulatory effect on fat mass and the obesity-associated gene FTO, which encodings the α-ketoglutarate-dependent dioxygenase FTO protein. Data from a previous genome-wide study has shown that the expression of miR-495 targets KLHL5 expression, and is positively associated with changes and subsequent organization involving platelet activation and aggregation in platelet microtubules and cytoskeleton in humans (14). In addition, several reports have suggested that inhibiting the expression of miR-495 can improve hemodynamics and angiogenesis in hypertensive conditions. Fu *et al* (23) demonstrated that the inhibition of miR-495 can promote hemodynamics and vascular remodeling, whose effects were involved in increasing angiogenic transcription factor (vascular endothelial zinc finger 1) and marked upregulation of angiogenic genes in pulmonary hypertension according to the analysis on the biological function of miR-495 in cultured pulmonary arterial endothelial cells under hypoxic conditions. Furthermore, attenuation of miR-495 has been documented to promote PTEN expression to protect against cardiomyocyte hypertrophy (24). It has also been reported that myocardial infarction-associated transcript, also known as retinal non-coding RNA 2 or Gomafu, can promote the progression of acute myeloid leukemia through suppression of miR-495 target genes (PBX3 and MEIS1) by not only promoting cell proliferation and cell cycle progression, but also decreasing apoptosis (25). In a study investigating the regulatory role of miR-200b in cor pulmonale model mice, miR-200b has been previously shown to potentially suppress protein kinase C (PKC)-α expression, which can modulate the proliferation and contraction of vascular smooth muscle cells evoked by the expression of endothelin 1 through the activation of protein kinases and transcription factors (11). The suppression of PKC-α can attenuate pathological fibrosis and restore cardiac function (26), indicating an association between miR-200b and protection against heart failure.

Analysis of the genotype combination for SNPs in the present study revealed that the TC/CC and CC/CC combined genotypes of miR-200bT>C and miR-495A>C polymorphisms were associated with susceptibility to HTN. Several previous studies have reported that members of the miR-200 family can promote the pathogenesis of atherosclerosis by targeting ZEB2 and RPS6KB1 gene expression to promote foam-cell formation (18,27,28). Although the present study did not cover the experimental evidence at the cellular level, Piperigkou *et al* (28) demonstrated that the difference in miRNA distributions implicated that miR-200b from five miRNA sequences of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141, and miR-429) might be involved in the regulation of epithelial-to-mesenchymal transition and cell proliferation, which can affect growth deformity and in turn the development of vascular diseases. A previous study into the role of miR-200b in the mechanism underlying stroke has reported that it can promote lipid accumulation whilst inhibiting cholesterol efflux by downregulating the expression of ATP binding cassette subfamily A member 1, an integrated membrane protein (29). When the expression of miR-200b was physiologically changed during hypoxia through manipulation of the miRNA level using miR-200b mimics and antagomirs, the expression of endothelial nitric oxide synthase (eNOS) was reduced, which limited the bioavailability of nitric oxide (NO) (18).

According to the haplotype results in the present study, the allele combination of miR-200bT>C and miR-495A>C in the haplotype of C-A significantly differed between the HTN and control groups. The regulatory miRNA SNPs in the promoter regions of miR-200b and miR-495 can affect the expression of their corresponding mature miRNAs, which can cause a significant impact on the expression of their target genes (14). The present study found interactive effects between miR-200b and miR-495 according to the SNP-SNP interaction analysis in a cohort of Korean patients. Through a combinatorial gene-environment analysis, Kim *et al* (20) previously reported that the combination of miR-495 CC genotype and low plasma folate contributed to an increased risk of rectal cancer according to a gene-environment combinatorial analysis. Using miRNA-miRNA co-expression profiling, Gatsiou *et al* (30) also found that there were possible associations between miRNA expression in platelets and their reactivity. Knockdown experiments revealed that miR-200b and miR-495 can target the expression of PRKAR2B and KLHL5, respectively. The regulatory subunit encoded by PRKAR2B is considered to control protein kinase A activity to regulate the activation and aggregation of platelets. When cAMP concentration increases, the catalytic subunit dissociates from the regulatory complex, to phosphorylate its target substrates, which suppresses platelet activation (31,32). The role of KLHL5, encoding a Kelch-like protein that binds actin and serves as a signaling molecule scaffold, in platelet function is reported to involve cytoskeletal reorganization and subsequent cell shape changes, which is an essential feature of platelet activation (16). According to these previous findings, it is possible that both miR-200b and miR-495 are involved in the activation of platelets, which have been reported to robustly influence the pathophysiology of coronary artery disease (33).

Based on the stratified analysis of the miR-200bT>C and miR-495A>C polymorphisms according to the HTN risk factors, the miR-200b TC+CC was associated with the BMI of <28.12 kg/m^2 and FBG levels of <106.26 mg/dl. By contrast, the miR-495 AA+CC was associated with HDL-C levels <44.29 mg/dl, whilst also being associated with SBP levels of ≥132.67 mmHg. These findings are similar to those of Motawi *et al* (21), who previously conducted a study on a cohort of Egyptian patients with pre-eclampsia and found a significant positive association between miR-495 expression and the parameters of systolic and diastolic blood pressure, and cholesterol level. In addition, a negative association was found with high density lipoprotein level and miR-495 expression (21).

The present study has several limitations. The study population was confined to a cohort of middle-aged Korean patients who visited the Jeju National University Hospital. The subjects, therefore, may not be representative of the general population from various contexts including age, ethnicity, place of residence and sampling period. For additional

verification, it will be necessary to conduct further studies on larger sample sizes containing more diverse patient cohorts. The functional mechanisms regarding how the miRNA polymorphisms may affect HTN remains unclear. Since the present study did not assess the expression levels of the miRNAs of interest on either molecular and functional levels, it is likely that the miR-200bT>C and miR-495A>C polymorphisms are associated with HTN risk by targeting the expression of genes involved in HTN induction. However, this requires experimental confirmation.

In conclusion, the present study investigated the relationship between HTN susceptibility and miR-200bT>C and miR-495A>C polymorphisms. It was found that the miR-495A>C polymorphism may be associated with HTN susceptibility. Although there have been studies describing the relationship between the miR-200 family and the incidence of tumorigenesis and cancer, the present study specifically demonstrated the association between the miR-200bT>C and miR-495A>C polymorphisms with HTN risk; to the best of our knowledge, this has not yet been reported. Therefore, results of the present study may provide evidence that miR-495A>C polymorphism is a potential candidate as a biomarker for HTN diagnosis and prevention in Korean populations.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

SHH designed the study, collected the medical data and performed the genetic analysis. YMC was a major contributor in analyzing and interpreting the data, and in writing the manuscript. All authors read and approved the final manuscript. YMC and SHH confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All enrolled subjects provided written informed consent to participate in the present study. The study was approved by the Institutional Review Board (approval no. JEJUNUH 2020-07-005) of Jeju National University Hospital (Jeju, Republic of Korea).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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