# Associations of leptin receptors and miRNA polymorphisms with susceptibility to hypertension

YOUNG REE KIM[1] and SEUNG-HO HONG[2]

[1]Department of Laboratory Medicine, School of Medicine, Jeju National University Hospital, Jeju 63241;
[2]Department of Science Education, Teachers College, Jeju National University, Jeju 63294, Republic of Korea

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**Abstract.** Leptin receptors (LEPR) are located in the central nervous system and other tissues including adipocytes and endothelial cells, where they play a key role in mediating the effects of leptin. MicroRNA (miR/miRNA)-27a and miR-155 have been shown to play an important role in the regulation of LEPR expression and are differentially expressed in various diseases. Therefore, the present study analyzed potential associations of LEPR deletion/insertion (Del/Ins), miR-27aA>G (rs895819) and miR-155T>A (rs767649) polymorphisms with a predisposition to hypertension (HTN). Genotyping was performed by a PCR-restriction fragment length polymorphism assay. Frequencies of LEPR Del/Ins and miRNA gene polymorphisms in patients diagnosed with HTN (n=232) and randomly selected healthy controls (n=247) were assessed. The present study found that Del/Ins and Ins/Ins genotypes and the Ins allele of the LEPR Del/Ins polymorphism were associated with a decreased risk of HTN compared with controls, whereas the miR-27aA>G rs895819 polymorphism was associated with an increased risk of HTN. Combined genotype and allele analyses for LEPR Del/Ins and two miRNA polymorphisms revealed an association with an increased risk or a decreased risk of HTN. Furthermore, stratification analysis revealed that HTN risk factors were associated with waist circumference (WC) and high-density lipoprotein cholesterol (HDL-C) values in LEPR Del/Ins polymorphism. They were also associated with body mass index, WC, triglyceride and HDL-C values in miR-27aA>G polymorphism. The present study revealed a combined effect of LEPR Del/Ins and miR-27aA>G polymorphisms on the risk of HTN in Koreans, suggesting that these gene polymorphisms could be potential markers for predicting HTN risk.

*Correspondence to:* Professor Seung-Ho Hong, Department of Science Education, Teachers College, Jeju National University, 61 Iljudong-ro, Jeju 63294, Republic of Korea
E-mail: shong@jejunu.ac.kr

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## Introduction

Hypertension (HTN) is one of the most common chronic diseases with an uncertain etiology. HTN is associated with cerebrovascular and cardiovascular diseases and mortality (1,2). It causes severe myocardial injury, heart failure, chronic kidney disease, peripheral vascular disease, stroke and derived complications (3-5). The complex pathogenesis of HTN is determined by an intricate combination of various genetic and environmental factors. Although several genetic factors show a variability of 25-60% in human HTN (4), the genetic mechanism of HTN remains uncertain.

Leptin is involved in the regulation of various cardiac and vascular events such as thrombosis, angiogenesis and cardiac hypertrophy (6). It also affects the regulation of metabolism, immunity and reproduction (7). Leptin has a physiological function through the leptin receptor (LEPR), a molecule distributed in various tissues that can mediate important effects of leptin as a hormone involved in whole-body energy homeostasis (8).

MicroRNAs (miRNAs/miRs) are small-sized noncoding RNAs consisting of nucleotides that mainly bind to 3'-untranslated regions (3'-UTRs) (9-11). They can either inhibit the translation of mRNA or accelerate its degradation. As biomarkers for several diseases, miRNAs have the potential to contribute to the understanding of pathogenesis mechanisms and the development of therapeutic medicine (4). As miRs are involved in various cellular processes including proliferation, differentiation, apoptosis and development, polymorphisms within miRs and the dysregulation of their expression are associated with metabolic diseases such as cancer, type 2 diabetes mellitus (T2DM), cardiovascular disease and gestational diabetes mellitus (12,13). For example, miR-27a shows a higher expression in the plasma of patients with hypertension and the omenta of obese patients (14). A study by Ciccacci *et al* (15) suggested that the G allele in miR-27a demonstrates a protective effect in T2DM, as overexpression of miR-27a in pre-adipocytes suppresses the expression of the peroxisome proliferator-activated receptor γ and the differentiation of adipocytes. MiR-155 expression levels in the aortae of adult spontaneous hypertensive rats are decreased and negatively correlate with blood pressure (16), suggesting that miR-155 might be involved in the development and pathological progression of HTN. Circulating miR-155 is

associated with T2DM and the rs767649 polymorphism in the pre-miR155 gene is associated with miR-155 expression (17).

The gene encoding LEPR is located on chromosome 1p31 (18). A deletion/insertion (Del/Ins) polymorphism located in the 3'-UTR of the LEPR gene may affect the rate of translation or degradation of mRNA by creating a stem-loop structure (19-21). Del/Ins polymorphism in the LEPR gene has mainly been reported to be associated with susceptibility to T2DM (19,22-27), although this remains controversial. Previous studies have also suggested that miR-27a and miR-155 play an important role in the regulation of LEPR expression (28,29). These miRNAs have also been found to be differentially expressed in various diseases.

Although the functions and gene expression of LEPR and miRNAs are known to be involved in susceptibility to various diseases, the effects of polymorphisms in LEPR and miRNAs genes on susceptibility to HTN have not yet been sufficiently elucidated. Thus, the present study performed a case-control study to investigate potential associations between LEPR 3'UTR Del/Ins, miR-27aA>G (rs895819) and miR-155T>A (rs767649) polymorphisms and HTN in a Korean population.

## Materials and methods

*Study population*. The present study included a total of 479 individuals, 232 patients with HTN, including 190 male and 42 female patients (mean ± SD age, 47.35±8.23 years; age range, 31-67 years), and 247 control subjects, including 132 male and 115 female subjects (mean ± SD age, 48.93±9.98 years; age range, 32-80 years), recruited from Jeju National University Hospital (Jeju, Korea) between January 2020 and December 2020. HTN was diagnosed after repeated measurements with a systolic blood pressure (SBP) >140 mmHg and a diastolic blood pressure (DBP) >90 mmHg. The present study also included subjects who were currently taking antihypertensive drugs. Patients who were diagnosed with other chronic diseases were excluded from the current study. Healthy control subjects had normal blood pressure and were not on medication, and were enrolled during the same period. The normal group was randomly selected from those who did not have chest pain, diabetes, hypertension or general illnesses as determined through physical examination. All study participants provided written informed consent. The study protocol was approved by the Institutional Review Board of Jeju National University Hospital, Republic of Korea (approval no. JEJUNUH 2020-07-005). The biospecimens and data used in this study were collected by the Biobank of Jeju National University Hospital, a member of the South Korea Biobank Network supported by the Ministry of Health and Welfare.

*Phenotype measurements*. The blood pressure of all participants was measured in a sitting position, after resting for at least 10 min. Risk factors for HTN were also measured. Body mass index (BMI) was calculated after weighing. Waist circumference (WC) was measured with a non-stretchable fiber measuring tape. All subjects fasted for >12 h before biochemical measurements. Levels of plasma fasting blood glucose (FBG) (cat. no. GLU CN R1 99118592, cat. no. GLU CN R2 99718692; FUJIFILM Wako Pure Chemical Corp.), triglycerides (TG; cat. no. TG CN R1 99333192, cat. no. TG CN R2 99933292; FUJIFILM Wako Pure Chemical Corp.) and high-density lipoprotein-cholesterol (HDL-C) (cat. no. ML HDLC S R1 55379, cat. no. ML HDLC S R2 55380; Minaris Medical Co., Ltd.) were measured with commercially available enzymatic colorimetric tests using an automatic biochemical analyzer (TBA 200FR NEO; Toshiba Medical Systems).

*Genotype analysis*. Genomic DNA was extracted from white blood cells using a G-DEX blood extraction kit (cat. No. 172R-1000; Intron Biotechnology, Inc.). Genotyping of LEPR Del/Ins, miR-27a (rs895819) and miR-155 (rs767649) polymorphisms were performed using a PCR and restriction fragment length polymorphism (RFLP) technique, as described in previous papers (28-30). Restriction enzyme digestion was performed at 37˚C for 17 h with *Rsa*I, *Dra*III and *Tsp*45I for LEPR Del/Ins, miR-27a (rs895819) and miR-155 (rs767649) polymorphisms, respectively (New England BioLabs, Inc.). Detailed PCR conditions and genotype sizes are summarized in Table I.

The following reference sequence shows the amplification region for analyzing the LEPR polymorphism (where bases in square brackets indicate the positions of the primers, the parentheses indicate pentanucleotide insertion and where * is the *Rsa*I cutting site): 5' [ATAATGGGTAATATAAAG TGTAATAGATT]*(ACTTT)AAAGTGTAATAGATTATA GTTGTGGGTGGGAGAGAGAAAAGAAACCAGAGT CAAATTTGAAAATAATTGTTCCAAATG[AATGTTGTC TGTTTGTTCTCT] 3'. It is truncated by *Rsa*I only in the case of the insertion genotype. However, the deletion genotype does not have pentanucleotide insertion and is not cleaved by *Rsa*I. The following sequence shows the amplification region for analyzing the miR-27a polymorphism (where bases in square brackets indicate the positions of the primers, where * is the *Dra*III cutting site): 5'-[GAACTTAGCCACTGTGAA CACGACTTGG]*GTGGACCCTGCTCACAAGCAGCTA AGCCCTGCTCCTCAGGCCAGGCACAGGCTTCGGGGC CTCTCTGCCACCCCGTCCCCGGGCAGCATCCTCGGT GGCAGAGCTCAGGGTCGGTTGGAAATCCCTGG[AAT GTGATTTGTGACAGGAAGCAA]-3'. The following sequence shows the amplification region for analyzing the miR-155 polymorphism (where bases in square brackets indicate the positions of the primers, where * is the *Tsp45*I cutting site): [5'-CCTGTATGACAAGGTTGTGTTTG]AGAACA AAAGGGGACCTGTGTGACATGTTTTATTTCAAT ATACATTTAGAGTTTGAACAAATAAAAAAAGCTATT AGAATTTTTAATATATATAACACATTATCAAAAACA CTG*CACTTTTCTGAGTGCTCTAATCAGGCAATT CGTATGTATATTTAAAAAGAGGGAAAAAGCTAATTA GTATACTAAATATATTTATTTTAATACCTGTGCTCA TATGTTATCTTTAAAGAACAGGAATAAAAT[TTATGG GTAGAATAGTATGCCAGC-3'].

*Statistical analysis*. P-values were calculated using a unpaired two-sided Student's t-test or Mann-Whitney U test for continuous variables and a $\chi^2$ test for categorical variables between the control subjects and the HTN group. Values are presented as mean ± standard deviation (SD) for clinical profiles of the control subjects and the HTN group. Logistic regression analysis adjusted for age and sex was applied to risk assessment with

Table I. Details of the SNPs (LEPR, miR-27aA>G and miR-155T>A) that were included in the present study.

| SNP | Primer sequence | PCR annealing, Tm./product (˚C/bp) | Genotype (bp) | RE |
|---|---|---|---|---|
| LEPR Del/Ins | 5'-ATA ATG GGT AAT ATA AAG TGT AAT AGA GTA-3'<br>5'-AGA GAA CAA ACA GAC AAC ATT-3' | 55˚C/114 | Del: 114<br>Del/Ins: 114, 90, 29<br>Ins: 90, 29 | *Rsa*I |
| miR-27a A>G | 5'-GAA CTT AGC CAC TGT GAA CAC GAC TTC G-3'<br>5'-TTG CTT CCT GTC ACA AAT CAC ATT G-3' | 55˚C/182 | AA: 155, 29<br>AG: 182, 155, 29<br>GG: 182 | *Dra*III |
| miR-155 T>A | 5'-CCT GTA TGA CAA GGT TGT GTT TG-3'<br>5'-GCT GGC ATA CTA TTC TAC CCA TAA-3' | 60˚C/294 | TT: 294<br>TA: 260, 155, 139<br>AA: 155, 138 | *Tsp*45I |

SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; RE, restriction enzyme; miR, microRNA.

LEPR Del/Ins, miR-27a (rs895819) and miR-155 (rs767649) polymorphisms for HTN. Values are presented as adjusted odds ratios (AOR) and 95% confidence intervals (95% CI). P<0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using GraphPad Prism version 4.0 (GraphPad Software, Inc.) and MedCalc version 12.7.1.0 (MedCalc Software Ltd.).

## Results

The HTN group had significantly higher BMI, WC, SBP, DBP, FBG and HDL-C values (P<0.0001) compared with the normal group. By contrast, HDL-C levels were significantly lower in the HTN group compared with in the normal group (P<0.001). However, age was not significantly different between the HTN and control group (P=0.09). The demographics of the HTN patient group and the control group are presented in Table II.

For the LEPR gene polymorphism, the genotypes were assessed as follows: A single 114 bp fragment for the wild-type homozygous alleles (Del genotype), two fragments of 90 and 24 bp for the mutated type (Ins genotype) and three fragments of 114, 90 and 24 bp for the heterozygous alleles (Del/Ins genotype) (Fig. 1). The 24 bp fragment is not shown in Fig. 1. For the miR-27aA>G polymorphism, homozygosity of the common allele (AA genotype) was represented by 155 and 27 bp bands, while the homozygosity of the variant allele (GG genotype) revealed itself as a 182 bp band. The heterozygous alleles (AG genotype) were revealed by 182, 155 and 27 bp bands (Fig. 2). The 27 bp fragment is not shown in Fig. 2. The lowest bands in lanes 1-6 are primer dimers. For the miR-155T>A gene polymorphism, the genotypes were assessed as follows: A single 294 bp fragment for the wild-type homozygous alleles (TT genotype), two fragments of 155 and 139 bp for the mutated type (AA genotype) and three fragments of 294, 155, and 139 bp for the heterozygous alleles (TA genotype) (Fig. 3).

Table II. Baseline characteristics between patients and control subjects.

| Characteristic | Controls (n=247) | HTN (n=232) | P-value |
|---|---|---|---|
| Age, years | 45.9±10.0 | 47.4±8.2 | 0.09 |
| BMI, kg/m$^2$ | 23.9±3.5 | 26.1±3.4 | <0.0001 |
| WC, cm | 81.3±8.7 | 87.2±9.2 | <0.0001 |
| SBP, mmHg | 118.5±11.2 | 138.4±13.4 | <0.0001 |
| DBP, mmHg | 71.4±8.6 | 88.5±8.8 | <0.0001 |
| FBG, mg/dl | 88.2±8.9 | 95.5±14.2 | <0.0001 |
| TG, mg/dl | 92.1±57.4 | 133.5±96.0 | <0.0001 |
| HDL-C, mg/dl | 57.5±14.3 | 52.2±11.3 | <0.0001 |

Values are presented as mean ± standard deviation (SD). HTN, hypertension; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglyceride; HDL-C, high density lipoprotein cholesterol.

Table III presents the comparison results of the genotype and allele frequencies of the LEPR Ins/Del, miR-27a A>G and miR-155 T>A polymorphisms between the HTN and control group. The LEPR Del/Ins and Ins/Ins genotypes and Ins allele were significantly associated with a decreased risk of HTN (Del/Del vs. Del/Ins: AOR=0.642, 95% CI 0.417-0.986, P=0.043; Del/Del vs. Ins/Ins: AOR=0.386, 95% CI 0.151-0.987, P=0.047; and Del allele vs. Ins allele: AOR=0.609, 95% CI 0.430-0.864, P=0.005). The frequency of the dominant model (Del/Del + Del/Ins vs. Ins/Ins) was also significantly lower in the case group compared with in the control group (AOR=0.597, 95% CI 0.397-0.898, P=0.013). The miR-27a AG

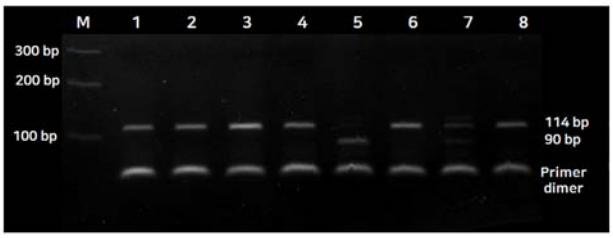


Figure 1. Restriction fragment length polymorphism results showing the restriction enzyme *Rsa*I digestion pattern of LEPR Del/Ins. M, 100-bp size marker; lanes 1, 2, 3, 4, 6 and 8, Del homozygous alleles; lane 5, Ins homozygous allele; lane 7, heterozygous allele (Del/Ins); LEPR, Leptin receptors; Del/Ins, deletion/insertion.

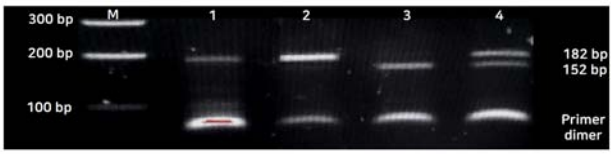


Figure 2. Restriction fragment length polymorphism results showing the restriction enzyme *Dra*III digestion pattern of miR-27a rs895819. M, 100-bp size marker; lane 5, wild-type homozygous allele (AA); lane 4, mutant-type homozygous allele (GG); lanes 1, 2 and 3, heterozygous alleles (AG); miR, microRNA.

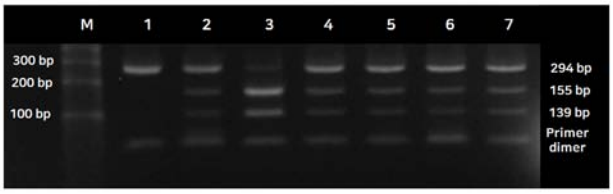


Figure 3. Restriction fragment length polymorphism results showing the restriction enzyme *Tsp45*I digestion pattern of miR-155 rs767649. M, 100-bp size marker; lane 1, wild-type homozygous allele (TT); lane 3, mutant-type homozygous allele (AA); lanes 2, 4, 5, 6 and 7, heterozygous alleles (TA); miR, microRNA.

genotype and dominant model were significantly associated with an increased risk of HTN (AA vs. AG: AOR=1.719, 95% CI 1.108-2.666, P=0.016; and AA + AG vs. GG: AOR=1.557, 95% CI 1.027-2.359, P=0.037). By contrast, the miR-155 T>A polymorphism was not significantly different between the patients with HTN and control subjects.

To investigate the association between genotype combination and the risk of HTN, the present study analyzed the combined genotypes of LEPR Ins/Del, miR-27a A>G and miR-155 T>A polymorphisms from the HTN and control groups. As shown in Table IV, the Del/Del-AG and Ins/Ins-AA combined genotypes (AOR=1.704, 95% CI 1.005-2.888, P=0.048; AOR=0.106, 95% CI 0.013-0.881, P=0.038, respectively) of the LEPR Del/Ins and miR-27a A>G polymorphisms and the AG-AA combined genotype (AOR=2.575, 95% CI 1.019-6.507, P=0.046) of the miR-27a A>G and miR-155 T>A polymorphisms could be linked with a genetic susceptibility to HTN.

To ascertain the synergistic effect between LEPR Ins/Del, miR-27a A>G and miR-155 T>A polymorphisms, an allele combination analysis was conducted (Table V). The results demonstrated that the Ins-A-A and Ins-G-T allele combination frequencies of the LEPR Ins/Del, miR-27a A>G and miR-155 T>A polymorphisms were significantly lower in the HTN group compared with in the control group (AOR=0.089, 95% CI 0.026-0.301, P<0.0001; AOR=0.403, 95% CI 0.204-0.800, P=0.038, respectively).

According to the stratified analysis results shown in Table VI, LEPR Del/Del vs. Del/Ins + Ins/Ins induced a decreased risk of HTN based on WC, FBG, and HDL-C values among the possible risk variables (WC<84.19 cm, P=0.044; FBG<91.72 mg/dl, P=0.044; HDL-C≥54.91 mg/dl, P=0.045). Whereas, miR-27a AA vs. AG + GG induced an increased risk of HTN based on BMI, WC, DBP, FBG, TG and HDL-C values among the possible risk variables (BMI<25.00 kg/m$^2$, P=0.046; WC<84.19 cm, P=0.021; DBP≥79.68 mmHg, P=0.019; FBG<91.72 mg/dl, P=0.036; TG<112.19 mg/dl, P=0.019; HDL-C<54.91 mg/dl, P=0.013).

## Discussion

According to a recent report, HTN affects 34.2 and 61.4% of the Korean population in the age ranges of >30 and 65 years of age, respectively (31). These facts led the current study to analyze potential associations of LEPR Del/Ins, miR-27a (rs895819) and miR-155 (rs767649) polymorphisms with a predisposition to HTN.

Regarding the LEPR 3'UTR Del/Ins polymorphism, the presence of a pentanucleotide insertion generates a putative stem-loop structure in the mRNA (19) and could affect the rate of degradation and/or translation of mRNA. In the present study, the LEPR Del/Ins and Ins/Ins genotypes as well as the Ins allele were significantly associated with a decreased risk of HTN. The WC, FBG, and HDL-C values were significantly decreased in Del/Ins + Ins/Ins carriers compared with in subjects with Del/Del. Previous studies have shown that the LEPR Del/Ins polymorphism is mainly associated with T2DM and its related traits (19,22-27). The results of the present study were consistent with the findings of the following studies, although the subjects of the present study were different. In particular, the results of the present study were similar to those of a Japanese study (26). In young Japanese men, plasma HDL-cholesterol and apoA-I levels were significantly lower in homozygous or heterozygous carriers of the Ins allele compared with in subjects homozygous for the Del allele. In another study, the results of LEPR Del/Ins polymorphism remained non-significant in morbidly obese French Caucasian families; however, subjects having a Del/Ins genotype showed a slight trend towards lower insulin values at 30 min after an oral glucose load compared with Del/Del individuals (24).

A total of three papers regarding the LEPR Del/Ins polymorphism have been published by a Finnish group (19,23,25). Serum insulin levels in morbidly obese subjects were found to be lower in heterozygous carriers of the Ins allele compared with in subjects homozygous for the Del allele (23). Individuals having a 3'UTR Del/Del genotype also had a slightly higher body weight throughout the study compared with those with an Ins allele in Finnish subjects showing impaired glucose tolerance (19). In addition, carriers of the Ins allele had a reduced risk of diabetes

Table III. Comparison of genotype frequencies of LEPR, miR-27a and miR-155 polymorphisms between patients with HTN and controls.

| Genotypes | Controls (n=247) | HTN (n=232) | AOR (95% CI) | P-value |
|---|---|---|---|---|
| LEPR Del/Ins | | | | |
| DD | 148 (59.9) | 172 (74.1) | 1.000 (reference) | |
| DI | 82 (33.2) | 53 (22.8) | 0.642 (0.417-0.986) | 0.043[a] |
| II | 17 (6.9) | 7 (3.0) | 0.386 (0.151-0.987) | 0.047[a] |
| Dominant | | | 0.597 (0.397-0.898) | 0.013[a] |
| Recessive | | | 0.434 (0.169-1.115) | 0.083 |
| Del allele | 378 (76.5) | 397 (85.6) | 1.000 (reference) | |
| Ins allele | 116 (23.5) | 67 (14.4) | 0.609 (0.430-0.864) | 0.005[a] |
| miR-27a A>G (rs895819) | | | | |
| AA | 84 (34.0) | 62 (26.7) | 1.000 (reference) | |
| AG | 109 (44.1) | 126 (54.3) | 1.719 (1.108-2.666) | 0.016[a] |
| GG | 54 (21.9) | 44 (19.0) | 1.193 (0.681-2.089) | 0.538 |
| Dominant | | | 1.557 (1.027-2.359) | 0.037[a] |
| Recessive | | | 0.869 (0.543-1.391) | 0.558 |
| A allele | 277 (56.1) | 250 (53.9) | 1.000 (reference) | |
| G allele | 217 (43.9) | 214 (46.1) | 1.152 (0.881-1.508) | 0.302 |
| miR-155 T>A (rs767649) | | | | |
| TT | 76 (30.8) | 70 (30.2) | 1.000 (reference) | |
| TA | 120 (48.6) | 113 (48.7) | 1.050 (0.675-1.633) | 0.828 |
| AA | 51 (20.6) | 49 (21.1) | 1.177 (0.687-2.017) | 0.553 |
| Dominant | | | 1.086 (0.721-1.635) | 0.693 |
| Recessive | | | 1.157 (0.724-1.851) | 0.542 |
| T allele | 272 (55.1) | 253 (54.5) | 1.000 (reference) | |
| A allele | 222 (44.9) | 211 (45.5) | 1.087 (0.831-1.422) | 0.543 |

[a]P<0.05. AOR was adjusted by age and sex of participants. AOR, adjusted odds ratio; CI, 95% confidence interval; HTN, hypertension; LEPR, Leptin receptors; miR, microRNA; Del/Ins, deletion/insertion.

compared with non-carriers (25). Similarly, in other groups, the Ins allele of the LEPR Del/Ins polymorphism is associated with a reduced risk of development of diabetes in Mexican (27) and Kashmiri (28) populations. These results suggest that alterations in the leptin signaling system can contribute to serum insulin levels and the development of T2DM. However, to the best of our knowledge, there is no study on the relationship between LEPR Del/Ins polymorphism and HTN. Therefore, the results of the present study need to be confirmed with larger samples from other hypertensive groups in the future.

MiRNAs are involved in the pathogenesis of HTN as well as various diseases such as cancer, coronary artery disease and diabetes (4). In particular, circulating miRNAs that target other miRNAs have been proposed to play a role in HTN in a tissue-based study (32). LEPR can interact with numerous miRNAs, including miR-27a and miR-155, which were selected in the present study. Of these two chosen miRNAs polymorphisms, the miR-27a AG genotype and dominant model of the present study was associated with an increased risk of HTN. The evidence for the involvement of miR-27a in hypertension includes that monocyte miR-27a in extracellular vesicles can decrease Mas receptor expression and eNOS phosphorylation in the endothelium, impair Ang-(1-7)-mediated vasodilation and cause hypertension (33). Another study has shown that a combination of mitoQ and endurance training has more prominent effects in improving cardiac health and ameliorating blood pressure in patients with HTN compared with mitoQ or endurance training alone through miR-27a modulation and amelioration of mitochondrial reactive oxygen species production (34). Plasma miRNA-27a also has a higher expression in the plasma of patients with HTN and the omenta of obese patients (14). Furthermore, miR-27a rs895819 polymorphism has been reported to alter disease susceptibility by interfering with the maturation and expression of miR-27a. The relationship between miR-27a polymorphism and disease susceptibility has been studied in gastrointestinal cancer (35), renal cell cancer (36), breast cancer (37) and myocardial infarction (38). To the best of our knowledge, the present study is the first to reveal the relationship between miR-27a rs895819 polymorphism and the risk of HTN.

In a previous study, miR-155 expression levels in the aortae of adult spontaneous hypertensive rats are decreased and negatively correlated with blood pressure (16), suggesting that miR-155 might be involved in the development and

Table IV. Genotype combination analysis for SNPs in patients with HTN and controls.

A, LEPR Del-Ins/miR-27a A>G

| Genotype combination | | Controls | HTN | | |
|---|---|---|---|---|---|
| SNP1 | SNP2 | (n=247) | (n=232) | AOR (95% CI) | P-value |
| DD | AA | 52 (21.1) | 48 (20.7) | 1.000 (reference) | |
| | AG | 64 (25.9) | 92 (39.7) | 1.704 (1.005-2.888) | 0.048[a] |
| | GG | 32 (13.0) | 32 (13.8) | 1.084 (0.558-2.106) | 0.812 |
| DI | AA | 21 (8.5) | 13 (5.6) | 0.874 (0.370-2.068) | 0.760 |
| | AG | 39 (15.8) | 31 (13.4) | 1.001 (0.526-1.905) | 0.999 |
| | GG | 22 (8.9) | 9 (3.9) | 0.626 (0.242-1.619) | 0.334 |
| II | AA | 11 (4.5) | 1 (0.4) | 0.106 (0.013-0.881) | 0.038[a] |
| | AG | 6 (2.4) | 3 (1.3) | 0.797 (0.166-3.831) | 0.777 |
| | GG | 0 (0.0) | 3 (1.3) | N/A | 0.998 |

B, miR-27a A>G/miR-155 T>A

| Genotype combination | | | Controls | HTN | |
|---|---|---|---|---|---|
| SNP1 | SNP2 | (n=247) | (n=232) | AOR (95% CI) | P-value |
| AA | TT | 21 (8.5) | 16 (6.9) | 1.000 (reference) | |
| | TA | 41 (16.6) | 34 (14.7) | 1.333 (0.567-3.137) | 0.510 |
| | AA | 22 (8.9) | 12 (5.2) | 0.967 (0.340-2.748) | 0.950 |
| AG | TT | 32 (13.0) | 39 (16.8) | 2.053 (0.851-4.955) | 0.109 |
| | TA | 57 (23.1) | 56 (24.1) | 1.591 (0.721-3.511) | 0.250 |
| | AA | 20 (8.1) | 31 (13.4) | 2.575 (1.019-6.507) | 0.046[a] |
| GG | TT | 23 (9.3) | 15 (6.5) | 1.274 (0.448-3.620) | 0.650 |
| | TA | 22 (8.9) | 23 (9.9) | 1.774 (0.660-4.770) | 0.256 |
| | AA | 9 (3.6) | 6 (2.6) | 1.452 (0.354-5.963) | 0.605 |

[a]P<0.05. AOR was adjusted by age and sex of participants. AOR, adjusted odds ratio; CI, 95% confidence interval; HTN, hypertension; LEPR, Leptin receptors; miR, microRNA; Del/Ins, deletion/insertion; SNP, single nucleotide polymorphism.

Table V. Comparison of allele combination between patients with HTN and controls (LEPR Del/Ins-miR-27a A>G-miR-155 T>A).

| Allele combination | Overall (n=958) | Controls (n=494) | HTN (n=464) | OR (95% CI) | P-value |
|---|---|---|---|---|---|
| Del-A-T | 0.2117 | 0.1963 | 0.2204 | 1.000 (reference) | |
| Del-A-A | 0.2307 | 0.2206 | 0.2512 | 1.021 (0.697-1.495) | 0.916 |
| Del-G-T | 0.2209 | 0.2092 | 0.2345 | 1.006 (0.683-1.482) | 0.974 |
| Del-G-A | 0.1457 | 0.139 | 0.1495 | 0.951 (0.616-1.468) | 0.821 |
| Ins-A-T | 0.0656 | 0.0781 | 0.0599 | 0.683 (0.390-1.195) | 0.180 |
| Ins-A-A | 0.0422 | 0.0656 | 0.0073 | 0.089 (0.026-0.301) | <0.0001[a] |
| Ins-G-T | 0.0498 | 0.0669 | 0.0305 | 0.403 (0.204-0.800) | 0.008[a] |
| Ins-G-A | 0.0334 | 0.0242 | 0.0468 | 1.743 (0.818-3.715) | 0.146 |

[a]P<0.05. HTN, hypertension; LEPR, Leptin receptors; miR, microRNA; Del/Ins, deletion/insertion; OR, odds ratio.

pathological progression of HTN. Nevertheless, in the present study, miR-155 (rs767649) polymorphism was not associated with HTN risk. However, since susceptibility to multifactorial diseases such as HTN is often indicated by a combination of multiple polymorphic variants rather than a single polymorphism, it cannot necessarily be said that a single polymorphism is unrelated. In the present study, Ins-A-A and Ins-G-T allele combinations of LEPR Ins/Del, miR-27a

Table VI. Stratified analysis of LEPR, miR-27a and miR-155 polymorphisms according to risk factors in patients with HTN.

| | LEPR Del/Ins + Ins/Ins | | miR-27a AG + GG | | *miR-155 TA + AA* | |
|---|---|---|---|---|---|---|
| Variables | AOR (95% CI) | P-value | AOR (95% CI) | P-value | AOR (95% CI) | P-value |
| BMI, kg/m$^2$ | | | | | | |
| <25.00 | 0.557 (0.306-1.012) | 0.055 | 1.921 (1.011-3.650) | 0.046 | 1.062 (0.590-1.910) | 0.842 |
| ≥25.00 | 0.722 (0.393-1.327) | 0.295 | 1.411 (0.787-2.529) | 0.248 | 0.918 (0.493-1.708) | 0.786 |
| WC, cm | | | | | | |
| <84.19 | 0.547 (0.304-0.984) | 0.044 | 2.034 (1.112-3.720) | 0.021 | 1.192 (0.661-2.151) | 0.559 |
| ≥84.19 | 0.615 (0.339-1.114) | 0.108 | 1.100 (0.594-2.039) | 0.761 | 1.005 (0.553-1.826) | 0.988 |
| SBP, mmHg | | | | | | |
| <128.16 | 0.726 (0.353-1.491) | 0.383 | 1.436 (0.683-3.019) | 0.340 | 1.132 (0.546-2.346) | 0.739 |
| ≥128.16 | 0.693 (0.335-1.432) | 0.322 | 1.838 (0.895-3.772) | 0.097 | 0.844 (0.397-1.796) | 0.660 |
| DBP, mmHg | | | | | | |
| <79.68 | 0.541 (0.238-1.230) | 0.143 | 1.214 (0.551-2.671) | 0.631 | 1.228 (0.560-2.692) | 0.609 |
| ≥79.68 | 0.640 (0.318-1.288) | 0.211 | 2.313 (1.148-4.661) | 0.019 | 0.646 (0.296-1.412) | 0.273 |
| FBG, mg/dl | | | | | | |
| <91.72 | 0.561 (0.321-0.983) | 0.044 | 1.901 (1.043-3.465) | 0.036 | 0.986 (0.567-1.715) | 0.959 |
| ≥91.72 | 0.743 (0.394-1.402) | 0.360 | 1.188 (0.633-2.230) | 0.592 | 1.011 (0.524-1.951) | 0.974 |
| TG, mg/dl | | | | | | |
| <112.19 | 0.646 (0.391-1.066) | 0.087 | 1.907 (1.114-3.264) | 0.019 | 1.245 (0.746-2.076) | 0.402 |
| ≥112.19 | 0.570 (0.270-1.204) | 0.141 | 1.196 (0.576-2.483) | 0.632 | 0.747 (0.350-1.593) | 0.450 |
| HDL-C, mg/dl | | | | | | |
| <54.91 | 0.660 (0.378-1.150) | 0.142 | 2.033 (1.161-3.561) | 0.013 | 0.925 (0.527-1.621) | 0.784 |
| ≥54.91 | 0.532 (0.287-0.986) | 0.045 | 1.116 (0.600-2.077) | 0.729 | 1.296 (0.700-2.400) | 0.410 |

Values are presented as mean ± standard deviation. The clinical parameters were divided by arithmetic mean in all participants. AOR was adjusted by age and sex of participants. AOR, adjusted odds ratio; CI, 95% confidence interval; HTN, hypertension; LEPR, Leptin receptors; miR, microRNA; Del/Ins, deletion/insertion; SNP, single nucleotide polymorphism; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglyceride; HDL-C, high density lipoprotein cholesterol.

A>G and miR-155 T>A polymorphisms were associated with a decreased risk of HTN. In addition, the Del/Del-AG combined genotype of LEPR Del/Ins and miR-27a A>G polymorphisms and the AG-AA combined genotype of miR-27a A>G and miR-155 T>A polymorphisms were also associated with the risk of hypertension. The combined analysis of alleles and genotypes of these polymorphisms can bring synergies that are difficult to obtain from a single polymorphism, which can be an important tool for research related to disease risk.

The current study had several limitations. The sample size of the study was relatively small. Hence, for additional verification, it will be necessary to conduct further studies on larger sample sizes containing more diverse patient cohorts.

In addition, there was no *in vitro* evidence to complement the genetic association study. Therefore, the study results warrant further functional studies to elucidate the mechanisms by which polymorphisms of LEPR Del/Ins, miR-27aA>G, and miR-155T>A affect HTN development.

In conclusion, the present study investigated the relationship between HTN susceptibility and LEPR Del/Ins, miR-27aA>G (rs895819) and miR-155T>A (rs767649) polymorphisms. The present study revealed that the LEPR Del/Ins and miR-27aA>G polymorphisms were associated with HTN susceptibility and that the combination of three polymorphisms was synergistic in analyzing HTN susceptibility. The present study could provide a basis for epidemiological studies of HTN and effective strategies for the early prevention of HTN in the Korean population. To the best of our knowledge, this is the first such study on HTN. The study results need to be confirmed in more diverse ethnic populations in the future. In addition, the study results require validation in laboratory-based functional studies.

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

**Ethics approval and consent to participate**

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

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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