Original Article

Association of Gln223Arg Polymorphism of the Leptin Receptor with Hypertensive Left Ventricular Hypertrophy

(leptin receptor / hypertension / polymorphism / left ventricular hypertrophy)

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Abstract. This study was aimed to determine whether the leptin receptor Gln223Arg polymorphism has an effect on plasma leptin levels and left ventricular hypertrophy in hypertension. The genotypes and allelic frequencies of the leptin receptor Gln223Arg were examined using the polymerase chain reaction and restriction fragment length polymorphism in 190 hypertensive patients and 88 healthy controls. Logarithmic (log) transformation of the plasma leptin level was carried out before performing comparison and regression analysis. The values of log(plasma leptin levels) in the hypertensive patients were higher than those of controls and they were higher in hypertensive patients with left ventricular hypertrophy than those without it (P < 0.05). The genotype (AA, AG, and GG) distribution of Gln223Arg polymorphism was 6.8, 33.8, and 59.4% in hypertensive patients with left ventricular hypertrophy, 4.3, 27.6, and 68.1% in patients without left ventricular hypertrophy, and 2.3, 26.1, and 71.6% in the controls, respectively. A significant difference was found among these three groups (P < 0.05). The frequency of allele A was higher in patients with left ventricular hypertrophy than in patients without it (23.6 vs. 18.1%; P < 0.05). Log(plasma leptin levels) and left ventricular mass index were higher in patients with the AA genotype than those with the AG or GG genotype in hypertensive patients (P < 0.05). In multivariate regression analysis, the AA genotype as an independent predictor had statistically significant effects on the left ventricular mass index. Our results suggest that the Gln223Arg polymorphism of the leptin receptor is significantly associated with plasma leptin levels and left ventricular hypertrophy in hypertension.

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Abbreviations: BSA – basic surface area, H-LVH – hypertension with left ventricular hypertrophy, H-NLVH – hypertension without left ventricular hypertrophy, IVST – interventricular septum thickness in diastole, LEPR – leptin receptor, LVDd – left ventricular end-diastolic diameter, LVH – left ventricular hypertrophy, LVM – left ventricular mass, LVMI – left ventricular mass index, PVST – posterior ventricular septum thickness in diastole.

Introduction

Left ventricular hypertrophy (LVH) is a complex trait and a common manifestation of heart impairment produced by hypertension. The percentage of patients with LVH in hypertension is 30–50 %, and LVH represents an independent risk of arrhythmia, heart failure and sudden cardiac death (Gradman and Altayoumi, 2006; Fabre and Sheppard, 2006). Moreover, it has been gradually proved that LVH is influenced by both genetic determinants and environmental factors. Heritability of echocardiographic LVH is around 30 % in large population studies (Post et al., 1997; Bella et al., 2004; Jin et al., 2011).

It is now well recognized that the role of leptin in energy homeostasis extends into development of cardiovascular diseases, including hypertension. Leptin, combined with the leptin receptor (LEPR), can activate the sympathetic nervous system both by local peripheral actions and through centrally mediated effects on the hypothalamus (Mark et al., 2009). In addition, leptin was shown to be involved in pathogenesis of obesity hypertension (Kshatriya et al., 2011). In recent years, studies exploring the relationship between LEPR polymorphism and familial combined hyperlipidaemia, early atherosclerosis, and metabolic syndrome have been carried out (van der Vleuten et al., 2006; Gottlieb et al., 2009; Saukko et al., 2010). Gln223Arg in exon 6 is one of the most interesting polymorphisms. However, little data is yet available on the impact of LEPR Gln223Arg polymorphism on hypertensive LVH. We performed a case-control study comparing the Gln223Arg polymorphism of LERP among healthy controls, hypertensive patients without LVH and hypertensive patients with LVH, and examined the association of this polymorphism with plasma leptin levels and left ventricular mass index (LVMI) in hypertensive patients.

Material and Methods

Subjects

The study was conducted in patients who attended the outpatient department of our institution with hypertension. The study participants consisted of 190 hypertensive patients and 88 age-matched healthy controls. The control participants had no cardiovascular or any other organ system disease, and had normal physical examination, chest roentgenogram, and two-dimensional and Doppler echocardiograms. Blood pressure was measured from the right arm using a standard mercury sphygmomanometer after 10 min of rest with the patient in the sitting position. Systolic blood pressure was measured at Korotkoff phase I and diastolic blood pressure at Korotkoff phase V, following the recommendations of the American Heart Association. The blood pressure was measured three times with an interval of not less than 30 s and the mean values were used for analysis. Hypertension was defined as a systolic blood pressure of greater than 140 mm Hg and/or a diastolic blood pressure of greater than 90 mm Hg on repeated measurements and/or receiving antihypertensive treatment. These hypertensive patients were divided into two subgroups: 74 cases in the H-LVH (hypertension with LVH) group, and the remaining 116 cases in the H-NLVH (hypertension without LVH) group according to the diagnostic criteria of echocardiographic LVH (left ventricular mass index (LVMI) values > 125 g/m^2 in males, > 110 g/m^2 in females). Exclusion criteria were secondary hypertension, diabetes mellitus, coronary artery disease (angina and/or electrocardiogram signs of ischaemia on treadmill-exercise test), two-dimensional wall motion abnormalities, valvular heart disease, cardiac arrhythmia or pacemaker implantation, New York Heart Association III or IV functional classes, cardiomyopathy, renal failure, hepatic failure, and lung diseases. The protocol was approved by the Hospital Ethics Committee and all participants gave their informed consent.

Echocardiography

All participants underwent complete transthoracic echocardiographic studies (Vivid 7, General Electric Company, Indianapolis, IN) using a 2.5–4.0 MHz transducer. An electrocardiograph was simultaneously recorded for every subject. Echocardiographic measurements were taken with participants in the left lateral decubitus position. Three consecutive cycles were averaged for every parameter. All examinations were performed by an experienced observer who had no knowledge of the participant's clinical information.

We measured left ventricular end-diastolic diameter (LVDd), interventricular septum thickness in diastole (IVST), and posterior ventricular septum thickness in diastole (PVST). Then the left ventricular mass (LVM) was calculated by using the anatomically validated formula: LVM (g) = $1.04(IVST+LVDd+PVST)^3 - (LVDd)^3 - 13.6$. LVMI was calculated as follows: body surface area (BSA) (m^2) = 0.06 × height (cm) + 0.0128 × weight (kg) - 0.1529 and then: LVMI (g/m^2) = LVM/BSA.

Laboratory measurement

A venous blood sample was collected from each participant under fasting conditions. Values of fasting blood glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglyceride were collected. The plasma leptin level was determined with a Human Leptin ELISA kit (NIBSC Code 97/594, BIO-EQUIP, Shanghai, China).

Determination of genotypes

Forearm venous blood samples were collected in 10-ml vacutainers containing EDTA. Genomic DNA was extracted from the cell pellet in whole blood using the Blood & Cell Culture DNA Mini Kit (Qiagen, Valencia, CA) and stored at −20°C until the genotyping was performed. The Gln223Arg polymorphism of the *LEPR* gene was determined by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using *Msp*I endonuclease enzyme restriction. The sense and anti-sense primers were 5'-TCCTCTTAAAAA-GCCTATCCAGTATTT-3', and 5'-AGCTAGCAAATA-TTTTTGTAAGCAAT-3'. Samples were amplified for 35 cycles consisting of denaturation at 94 °C for 30 s, annealing at 55 °C for 90 s, and extension at 72 °C for 45 s, and then followed by a final extension step at 72 °C of 10 min. The Gln223Arg polymorphism introduced a restriction site that cut the 421-bp PCR amplicon into 294-bp and 127-bp fragments. Digested PCR fragments were separated electrophoretically in 2% agarose gels.

Statistical analysis

All the analyses were carried out using the SPSS statistical package, version 13.0 (SPSS Inc., Chicago, IL). All data were expressed as the mean ± SD. All frequencies were estimated by gene counting. The Hardy-Weinberg equilibrium analysis was performed using the χ^2 comparison. To investigate the difference of Gln223Arg polymorphism of the *LEPR* gene, we compared the allele and genotype frequencies by the χ^2 test and by post-hoc Tukey's test for multiple comparisons among healthy controls, hypertensive patients without LVH and those with LVH. Because leptin levels showed skewed distributions, logarithmic (log) transformation was carried out before performing statistical analysis. We studied the clinical parameters by *LEPR* genotypes using analysis of covariance with adjustments for age, gender and BMI. In addition, we also performed multivariate linear regression analysis to evaluate the effects of *LEPR* genotypes on the echocardiographic index, LVMI. A P value of < 0.05 was considered to be statistically significant for all analyses.

Results

The characteristics of the individuals investigated are shown in Table 1, and these data were separated to the men and women cohort in every group. Compared with the control group, the patients with hypertension had higher BMI, blood pressure values, hypertension duration and log(plasma leptin level) ($P < 0.05$). The hypertensive patients with LVH had significantly higher log(plasma leptin level) than those without LVH ($P < 0.05$).

The distribution frequency of genotypes and alleles of LEPR of the three groups is shown in Table 2. The genotype frequencies were as follows: 2.3 % (AA), 26.1 % (AG) and 71.6 % (GG) in the control group, 4.3 % (AA), 27.6 % (AG) and 68.1 % (GG) in hypertensive patients without LVH, and 6.8 % (AA), 33.8 % (AG) and 59.4 % (GG) in hypertensive patients with LVH. The observed genotype frequencies conformed to Hardy-Weinberg's equilibrium. The genotype and allele frequencies of the LEPR Gln223Arg polymorphism were significantly different among the three groups ($P < 0.05$). The frequency of allele A was higher in the hypertensive groups than in the controls, and the patients with LVH had higher allele A frequency than those without LVH ($P < 0.05$).

In the analysis of covariance adjusted for age, gender and BMI, the patients having the homozygous AA variant had significantly higher log(plasma leptin level) and LVMI than those with the GG/AG genotype ($P < 0.05$), whereas BMI, SBP, DBP, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride and fasting glucose were not significantly higher ($P > 0.05$) (Table 3).

Furthermore, in the multivariate regression analysis model for age, gender, BMI, duration of hypertension,

Table 1. Comparison of biological and biochemical variables among healthy controls, hypertensive patients without LVH and those with LVH

	Controls (N = 88)	**Hypertension without LVH (N = 116)**	**Hypertension with LVH (N = 74)**
Male/Female	46/42	63/53	41/33
Age (years)	52.2 (9.6)	55.7 (8.1)	56.4 (8.5)
Male	51.3 (10.9)	54.4 (7.9)	55.6 (9.1)
Female	53.2 (8.4)	57.1 (9.2)	57.5 (9.6)
BMI (kg/m^2)	23.8 (3.5)	25.2 (4.3)*	26.7 (4.8)*
Male	24.5 (4.7)	26.1 (5.0)*	27.2 (5.3)*
Female	23.0 (5.2)	24.7 (5.6)*	25.1 (3.9)*
Total cholesterol (mg/dl)	178.7 (18.1)	191.4 (20.1)	204.5 (21.0)
Male	182.4 (21.9)	203.2 (19.4)	199.1 (18.3)
Female	173.4 (16.8)	184.6 (18.7)	208.3 (19.5)
LDL cholesterol (mg/dl)	118.3 (17.2)	123.1 (16.7)	115.8 (19.3)
Male	120.6 (18.4)	117.5 (15.9)	119.1 (21.3)
Female	115.7 (16.3)	129.8 (17.2)	110.8 (18.6)
HDL cholesterol (mg/dl)	45.2 (9.8)	46.8 (10.3)	47.1 (11.4)
Male	47.1 (10.7)	49.2 (11.4)	48.6 (12.3)
Female	44.0 (10.2)	44.3 (10.5)	45.6 (11.7)
Triglyceride (mg/dl)	155.3 (19.2)	140.7 (18.1)	146.9 (17.9)
Male	161.2 (20.8)	151.9 (19.4)	149.3 (18.2)
Female	152.4 (21.6)	134.2 (17.5)	144.7 (16.3)
Fasting glucose (mg/dl)	94.2 (8.1)	96.9 (7.2)	98.1 (8.2)
Male	99.2 (10.2)	93.5 (8.9)	99.6 (10.8)
Female	91.3 (9.4)	98.0 (7.1)	95.7 (8.5)
SBP (mmHg)	122.1 (12.7)	147.2 (11.6)*	151.4 (10.6)*
Male	118.4 (13.5)	150.6 (12.4)*	154.2 (9.3)*
Female	125.2 (11.6)	143.9 (11.3)*	147.5 (10.7)*
DBP (mmHg)	76.3 (4.8)	85.8 (7.2)*	87.2 (9.1)*
Male	78.1 (5.3)	87.3 (8.1)*	85.1 (9.4)*
Female	73.6 (5.6)	82.4 (7.9)*	88.9 (7.2)*
Duration of hypertension (years)	0	6.3 (2.6)	8.1 (3.2)*#
Male	0	5.8 (3.1)*	8.8 (3.6)*#
Female	0	6.9 (2.7)*	7.4 (2.9)*
Log(leptin)(ng/ml)	0.68 (0.24)	0.77 (0.31)*	1.15 (0.29)*#
Male	0.72 (0.31)	0.84 (0.36)*	1.21 (0.31)*#
Female	0.61 (0.26)	0.72 (0.28)*	1.07 (0.23)*#

Values are expressed as mean ± SD or numbers. Probabilities were determined by one-way analysis of variance or χ^2 test.
SBP – systolic blood pressure; DBP – diastolic blood pressure. Compared with the control group, *$P < 0.05$; compared with hypertensive patients without left ventricular hypertrophy ,#$P < 0.05$

Table 2. Genotype and allele frequencies of the LEPR Gln223Arg polymorphism among healthy controls, hypertensive patients without LVH and those with LVH

Group	N	Genotype N (%)			Allele N (%)	
		AA	AG	GG	A	G
Controls	88	2 (2.3)	23 (26.1)	63 (71.6)	27 (15.3)	149 (84.7)
H-NLVH	116	5 (4.3)*	32 (27.6)	79 (68.1)	42 (18.1)	190 (81.9)
H-LVH	74	5 (6.8)*,#	25 (33.8)*	44 (59.4)*	35 (23.6)*,#	113 (76.4)*

Compared with the control group, *P < 0.05; compared with hypertensive patients without left ventricular hypertrophy, #P < 0.05

SBP, DBP and *LEPR* genotype as independent variables and for LVMI as dependent variable, the female gender (β = 0.127, P = 0.036), BMI (β = -0.021, P = 0.024), duration of hypertension (β = 0.198, P = 0.037), and LEPR Gln223Arg (AA vs. AG+GG) (β = 0.171, P = 0.019) had statistically significant effects on LVMI as independent predictors, respectively (Table 4).

Discussion

In our study, we observed that there was a significant difference in the log(plasma leptin level), genotype and allele distribution of LEPR Gln223Arg among healthy controls, hypertensive patients with LVH and those without LVH. AA homozygotes of the LEPR Gln223Arg polymorphism had significantly higher LVMI and higher plasma leptin levels than the G-allele carriers. Furthermore, the LEPR Gln223Arg polymorphism was associated with LVMI in hypertensive patients with adjustments for age, gender, BMI and duration of hypertension. Our results suggest that the Gln223Arg polymorphism of LEPR is significantly associated with plasma leptin levels and LVH in hypertension.

Leptin, the product of the obese gene, is primarily secreted by adipose tissue and it regulates body weight by its receptor-mediated anorectic, thermogenic, and antisteatotic effect (Morton et al., 1999). Leptin mediates energy homeostasis through hypothalamic pathways, and it seems to serve primarily as a signal of sufficient energy for the human body (Friedman and Halaas, 1998). If an individual starts to receive less nutrition and loses weight, the leptin levels will decrease, and concomitantly, through a mechanism of physiologic adaptation, appetite will increase and energy expenditure will decline (Bjorbaek and Kahn, 2004). Therefore, leptin levels most often accompany obesity, likely representing a state of leptin resistance (Considine et al., 1996).

Table 4. Multivariate regression analysis for the association between the LEPR genotypes and LVMI

	LVMI	
	β	P
Age	–0.035	0.142
Gender	0.127	0.036
BMI	–0.021	0.024
Duration of hypertension	0.198	0.037
SBP	0.186	0.061
DBP	0.104	0.094
LEPR Gln223Arg	0.171	0.019

SBP – systolic blood pressure; DBP – diastolic blood pressure; β values were derived from the multivariate regression analysis model adjusted for age, gender, body mass index, duration of hypertension, systolic and diastolic blood pressure and represented standardized regression coefficients; gender, female vs. male; LEPR Gln223Arg,AA vs. AG+GG.

In addition, leptin was shown to be an independent risk factor for ischemic heart disease in the West of Scotland Coronary Prevention Study (WOSCOPS) (Tune and Considine, 2007) and increased in congestive heart failure (Konstantinides et al., 2001). It was also demonstrated that leptin increase was correlated with left ventricular mass in hypertensive subjects (Sierra-Honigmann et al., 1998). Most of these links have been ascribed to an interaction between leptin and sympathetic activation, pressor effects (Mark et al., 2009), enhancement of platelet aggregation (Tune and Considine, 2007), impairment of fibrinolysis (Konstantinides et al, 2001), pro-angiogenic actions (Sierra-Honigmann et al., 1998) and systemic inflammation (Romero-Corral

Table 3. Clinical characteristics in relation to Gln223Arg polymorphism in hypertensive patients

	AA	AG	GG
SBP (mmHg)	148.6 (9.6)	153.5 (11.8)	147.8 (11.2)
DBP (mmHg)	88.4 (8.3)	84.8 (9.1)	86.3 (7.8)
Total cholesterol (mg/dl)	193.4 (21.6)	203.4 (20.6)	205.7 (21.1)
LDL cholesterol (mg/dl)	126.7 (14.9)	122.2 (16.3)	116.7 (15.8)
HDL cholesterol (mg/dl)	48.1 (8.2)	45.4 (9.1)	47.3 (11.5)
Triglyceride (mg/dl)	144.6 (9.6)	143.9 (10.4)	147.4 (11.2)
Fasting glucose (mg/dl)	96.6 (5.2)	99.1 (4.6)	98.5 (5.7)
Log(leptin)(ng/ml)	1.23 (0.37)*,#	1.01 (0.28)	0.89 (0.26)
LVMI (g/m^2)	126.3 (11.5)*,#	111.7 (10.3)	102.0 (9.5)

Values are expressed as mean ± SD or numbers. Probabilities were determined by one-way analysis of variance. The analyses were adjusted for age, gender and body mass index. DBP – diastolic blood pressure; compared with the GG group, *P < 0.05; compared with the AG group, #P < 0.05

et al., 2008). In our study, we found that the values of log(plasma leptin level) in hypertensive patients were higher than those of controls, and there was also a difference between the hypertensive subgroups, implying that elevated leptin levels were associated not only with hypertension, but also with hypertensive LVH. Previous studies in transgenic animals and in cell culture have reported that leptin may play a role in determining LVH (Barouch et al., 2003; Rajapurohitam et al., 2003; Tajmir et al., 2004). Perego et al. (2005) revealed that leptin also contributed to the LVH in humans using immunocytochemistry of human heart biopsies. Indeed, leptin can induce proliferation, differentiation, and functional activation of haemopoietic and embryonic cells to promote myocyte growth (Umemoto et al., 1997). Moreover, in rats with myocardial infarction, cardiac hypertrophy is attenuated by blockade of LEPR (Purdham et al., 2008). Among the suggested mechanisms of leptin-induced hypertrophy are the stimulation of endothelin-1, angiotensin II (Rajapurohitam et al., 2006), and reactive oxygen species (Nagae et al., 2009). Hypertension is one of the most common diseases leading to LVH, but at present the exact mechanism of how elevated leptin levels have a contributory effect on hypertensive LVH is unclear, and further experiments and clinical trials are needed.

LEPR is a single-transmembrane-domain receptor of the cytokine-receptor family with widespread distribution and several alternatively spliced isoforms (one long isoform and several short isoforms), one of which is the soluble LEPR present in blood. In lean subjects, the largest part (60–98 %) of leptin in the blood is present in the bound form; however, in obese subjects the majority of leptin is unbound (Sinha et al., 1996). LEPR is also present in peripheral tissues such as pancreatic β cells, liver, and skeletal muscle. In these tissues, leptin is capable of stimulating lipid oxidation via LEPR with a subsequent increase in insulin sensitivity (Muoio et al., 1997; Shimabukuro et al., 1997). The human *LEPR* gene is located on chromosome 1p31 and several single-nucleotide polymorphisms have been described in this gene. The Gln223Arg polymorphism is located within the region encoding the extracellular domain of LEPR and therefore present in all isoforms of the receptor. Therefore, the amino-acid change affects all forms of the receptor and may change its functional characteristics. The homozygous 223AA genotype of LEPR was found to be associated with a lower binding capacity of leptin to the soluble form of the receptor in plasma, which was interpreted as indicating abnormal receptor function (Stefan et al., 2002). Our results revealed that the Gln223Arg polymorphism of LEPR was significantly associated with hypertensive LVH and higher log(plasma leptin level). Earlier epidemiologic studies have demonstrated that the A allele of the LEPR Gln223Arg polymorphism was related to several detrimental processes or outcomes in some populations – for example, it was related to metabolic syndrome in free-living community elderly people in the south Brazil region (Gottlieb et al., 2009), relevant to higher risks of hypertension after adjustment for age and sex in a Chinese population (Gu et al., 2012), and associated with macroangiopathy in Chinese type-2 diabetes mellitus (Gan and Yang, 2012). Gottilieb et al. (2009) reported that there were differences in genotype frequencies, consisting in reduction in the GG genotype and an excess of the AA genotype in the metabolic disorder group and metabolic syndrome group compared to the healthy control group, and they showed that the association between metabolic syndrome and the Gln223Arg LEPR polymorphism was independent of sex, age and other metabolic disorders.

Gu et al. (2012) found that the AA genotype and A allele of Gln223Arg caused higher leptin levels and DBP. All these phenomena impelled investigators to search for the mechanisms and pathways involved. It was hypothesized that the 223AA genotype might cause impairment in the lipid metabolism through leptin signalling. Another study reported that the A allele of the *LEPR* gene may contribute to the low plasma levels of high-density lipoprotein by modifying hepatic lipase, phospholipid transfer protein, cholesteryl ester transfer protein, or lipoprotein lipase (Soro et al., 2003). In 2005, a genome-wide linkage analysis in hypertensive siblings indicated that a locus for high-density lipoprotein levels was located on chromosome 1p, to which the *LEPR* gene has also been mapped (Kullo et al., 2005). As mentioned above, the 223AA genotype of *LEPR* results in a lower binding capacity of leptin to the soluble form of the receptor in the plasma, and may also be an important link between the Gln223Arg polymorphism and the detrimental clinical manifestation, including hypertensive LVH. Our study was just a clinical investigation and we hypothesized that the 223AA genotype of LEPR would lead to a lower binding capacity of leptin to LEPR, thus causing higher serum free leptin levels. Then hyperleptinaemia would activate the sympathetic nervous system, increase the vascular tone and impair renal sodium excretion to produce hypertension (Kshatriya et al., 2011). Gradually, the increased leptin levels resulted in LVH both through neuropeptide activation and other proliferation promoter effects. These hints of genetic determinants inspired us to take further steps to explore the potential underlying mechanisms.

Conversely, there were also opposing findings revealing that the G allele of LEPR Gln223Arg was associated with increased adiposity, BMI and percent fat mass as well as circulating insulin and leptin levels (Enns et al., 2011). Saukko et al. (2010) found that there was no significant difference in the leptin concentration among LEPR Gln223Arg genotypes when adjusted for sex, age, and BMI. Besides, it was reported that there was no association between LEPR Gln223Arg polymorphism and the relative weight or lipid profile in Japanese children (Endo et al., 2000), or in Turkish children (Komşu-Ornek et al., 2012) or Polish children (Pyrzak et al., 2009). The reasons for these controversial results might probably be that the investigated populations

were of different races or from different regions, with different sample sizes having different statistical power.

Our present study has some limitations. First, our sample size is relatively small. Second, as our study is a case-control study, it has less external validity than a random population sample. Further studies with larger sample sizes are needed to confirm our results.

Conclusion

Our data suggest that the *LEPR* gene 223A>G polymorphism was significantly associated with the occurrence of LVH in hypertensive patients. Also, we found that the 223A>G polymorphism of the *LEPR* gene affected plasma leptin concentrations in hypertensive patients. This study provides new clinically relevant information regarding the genetic determinants modulating hypertensive LVH. Additional studies are warranted to confirm our findings and clarify the mechanisms involved.

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