

# Left ventricle geometry, echoreflectivity and CYP11B2 polymorphism C-344T in hypertensive males

Lozinska Marina, Zhebel Vadym, Lozinsky Sergiy\*

## ABSTRACT

**Objectives:** Hypertensive heart remodeling requires the assumption of different factors, including an increase of left ventricular mass (LVM) and myocardial fibrosis. It was shown that aldosterone stimulates cardiac collagen synthesis and fibroblast proliferation. *CYP11B2* is one of the genes responsible for the effects of aldosterone. Therefore, hypertensive remodeling could be partially related to the polymorphism of this gene. The purpose of this study was to assess the association of *CYP11B2* polymorphism with structural remodeling by changes in geometry and myocardial density to define their role and interaction in hypertensive heart disease. **Methods:** The study recruited 150 men aged 45-60 with and without essential hypertension (EH), who possessed no irreversible target organ damages. Fifty of them had normal BP, 58 had EH without left ventricular hypertrophy (LVH) and 42 had EH and LVH. Each participant underwent office blood pressure measurement, echocardiography with echo-reflectivity analysis, and determination of the C-344T polymorphism of the aldosterone synthase gene *CYP11B2*. **Results:** Patients with EH and LVH differed not only by LV mass but also by larger body mass, relative wall thickness, and wider echo-reflectivity spectrum. The associations of larger end diastolic diameter with C allele, and the larger thickness of the posterior wall and interventricular septum with T allele, were revealed only in patients with EH and LVH. **Conclusions:** Hypertensive patients with LVH are likely to be a distinct cluster with their own genetic predisposition to hypertensive heart disease.

**Key words:** essential hypertension, hypertensive heart remodeling, myocardial fibrosis, echo-reflectivity, CYP11B2 polymorphism

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## INTRODUCTION

Aldosterone plasma activity plays an important role in the general homeostasis. The retention of water and electrolytes is a common mechanism of blood pressure up-regulation that impacts not only BP level, but also myocardial remodeling. LVH is the most common sequelae of hypertension and is a result of cardiac remodeling and hypertrophy. It is a strong risk factor for cardiovascular events and all-cause mortality, especially arrhythmias and sudden cardiac death<sup>1</sup>. Hypertensive heart remodeling involves different processes. An increase of left ventricular mass (LVM) is the most popular event beside myocardial fibrosis. It has been shown that aldosterone stimulates cardiac collagen synthesis and fibroblast proliferation via activation of local mineralocorticoid receptors in the heart<sup>2</sup>. *CYP11B2* is one of the genes responsible for the effects of aldosterone, thus, hypertensive remodeling could be partially related to the polymorphism of this gene. Up to date, we have no reliable methods of estimation of fibrosis, and the question of how it contributes to the phenotype of hypertensive heart disease remains unexplored. We tried to assess the association of *CYP11B2* polymorphism with structural

remodeling by changes in geometry and myocardial density, aiming to define their role and interaction in hypertensive heart disease.

## METHODS

The protocol of the study was approved by the local ethics commission of Vinnitsia Regional Dispensary of Radiation Defense of Population. The informed consent was mandatory for all participants. The study involved men aged 45-60 years old, with or without essential hypertension (EH), who had no irreversible target organ damages. Diagnosis of hypertension was established according to the ESC recommendations (2013). The group of normotensive persons, including men of the same age with normal blood pressure and without any pathological changes detected by echocardiography (ECG). The age criteria covered most patients with hypertension. The criteria for exclusion from the study were as follows:

- Congenital or acquired heart defects
- Systemic connective tissue diseases
- Endocrine diseases

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- Chronic kidney disease
- Secondary essential hypertension
- Myocardial disease and heart failure not associated with essential hypertension
- Pulmonary essential hypertension
- Hemodynamically significant arrhythmias (atrial fibrillation or flutter, AV blocks of 2<sup>nd</sup> and 3<sup>rd</sup> degree, high-grade premature beats)
- Unsatisfactory ultrasound visualization of the heart
- DM of 1 type or uncontrolled DM of type 2
- Unstable or variant angina on the day of inclusion.
- Specific oligonucleotide primers: 5'- CAG GAG GAGACC CCA TGT GAC -3'; 5'- CCT CCA CCC TGT TCA GCC C -3';
- 2.5  $\mu$ L of 10X buffer for amplification;
- 2 mM of magnesium chloride
- 0.2 mM of mixture of deoxynucleotide triphosphates (dNTP)
- 2.5 units of Taq DNA polymerase
- 20-50 ng of genomic DNA
- 25  $\mu$ L of mineral oil was poured into the test tubes

The patients involved in the study did not receive regular antihypertensive therapy. Each participant underwent the following activities: office blood pressure measurement (according to the recommendations by the ESC), echocardiography with echo-reflectivity analysis, and determination of the polymorphism of the aldosterone synthase gene *CYP11B2*.

Basic measurements of left ventricle such as end diastolic and end systolic dimensions (EDD and ESD), left ventricular mass indexed by the body area (LVMI), ejection fraction (EF), relative wall thickness (RWT) were obtained by echocardiography. Echocardiography had been performing using Sigma 5000 equipment (Kontron Medical, France). We located 2 representative areas 2010 mm in size, one in the interventricular septum, and another in an LV posterior wall, on the images in the parasternal long axis view and saved them for the future processing. During the procession, the broadband (BB) of the black and white spectrum of chosen regions of interest was calculated by ImageJ software in an offline mode. **Figure 1** demonstrates an example of such processing of the images. According to available resources, this parameter is the most reliable echo-reflectivity marker of myocardial fibrosis. In an experimental study, its correlation with collagen volume fraction measured by biopsy was as high as 0.72 ( $p=0.03$ )<sup>3</sup>.

The polymorphism of the *CYP11B2* gene was determined from venous blood samples of all participants by PCR method. Blood genomic DNA was extracted using a set of reagents purchased from LLC NPF Synthol, Russia. Polymorphic regions of *CYP11B2* gene were amplified by a polymerase chain reaction. The final volume of the reaction mixture was 25  $\mu$ L and consisted of:

The amplification was performed on the amplifier "Tertsik" (LLC "DNA-Technology", Russia), using the amplification program, which started with a denaturation at 94<sup>o</sup>C for 5 minutes, following by 35 cycles of amplification. For the identification of the alleles, we carried out a restrictive analysis of the amplicons, using endonuclease restriction HaeIII (SibEnzyme, Russia) at 37<sup>o</sup>C.

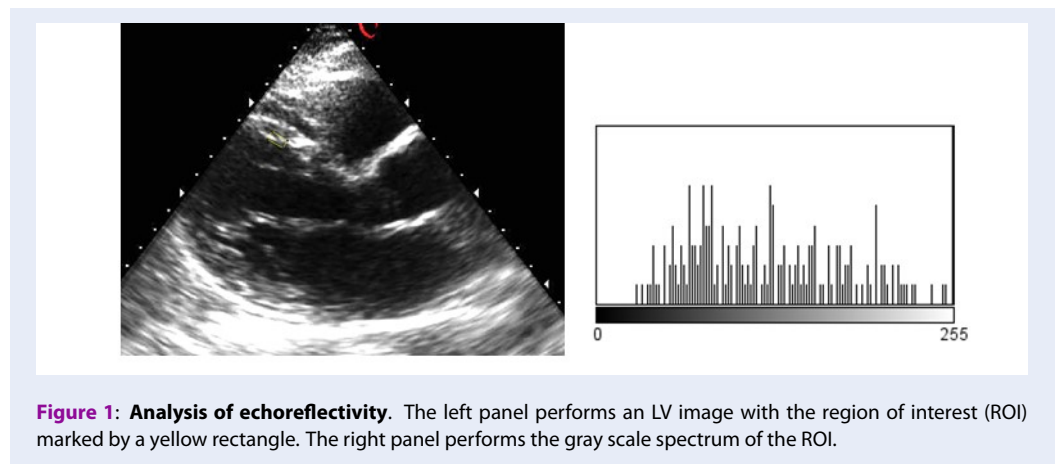
The cleavage products of the polymorphic sites of *CYP11B2* gene were detected by electrophoresis in 5% agarose gel (Agarose SFR, AMRESCO, USA), in a single TBE buffer (50 mM Tris-H<sub>3</sub>BO<sub>3</sub> and 2 mM EDTA, pH 8.0) for 1 hour at 3 – 4 V per 1 cm of gel. Pbr322/AluI was used as DNA ladders. Gels were stained with ethidium bromide, followed by visualization of results under UV-light.

## RESULTS

A total of 50 men with normal blood pressure and 100 patients with EH were examined. Among those, 58 participants had hypertension without any damages of the target organs and all had normal LVM. Other 42 patients had hypertension with the ECG signs of LV hypertrophy, established on the values of LVMI > 110g/m<sup>2</sup>, or had at least one of the LV walls exceeded 12mm in thickness. The results of anthropometry and echocardiography are presented below (**Table 1**).

All groups did not differ in their ages and height. However, group 2 and 3 showed higher body mass compared to group 1 (**Figure 2**). This observation was the same for the SBP and DBP parameters (**Figure 3**). LVM differed dramatically: the indexed means LVM were almost the same in the 1<sup>st</sup> and 2<sup>nd</sup> groups, while in the 3<sup>rd</sup> group, they were much higher. Changes of RWT were similar in all groups (**Figure 4**).

Differences in LV dimensions and ejection fraction between groups were not significant. The results of



**Table 1: Anthropometry and echocardiography measurements in groups 1, 2 and 3**

	NT (Group 1), M (SD) n=50	EH (Group 2), M (SD) n=58	EH+LVH (Group 3), M (SD) n=42
Mean age, y.	52.5 (5,0)	51.8 (5,3)	52.3 (5,3)
Height, sm	174.3 (12,3)	176.4 (8,5)	177.4 (4,8)
Weight, kg	81.4 (13,2)	95.3 (21,2)	101.9 (15,5)
SBP, mm Hg	124.4 (15,8)	151.6 (19,4)	160.2 (21,8)
DBP, mm Hg	75.1 (9,9)	92.8 (11,6)	94.7 (13,3)
EDD, mm	48.6 (5,2)	48.9 (5,2)	52.1 (5,6)
ESD, mm	32.7 (4,1)	33.3 (4,5)	34.3 (5,6)
EF, %	61.2 (7,7)	59.9 (7,7)	59.7 (10,6)
LVM, g/m <sup>2</sup>	82.3 (16,9)	82.9 (18,7)	131.4 (25,5)
RWT	0.39 (0,06)	0.41 (0,07)	0.52 (0,11)

NT – normal BP; EH –essential hypertension; EH+LVH – essential hypertension and left ventricularhypertrophy; SBP – systolic blood pressure; DBP – diastolic blood pressure; EDD– end-diastolic dimension; ESD – endsystolic dimension; EF – ejection fraction; LVM – left ventricular mass; RWT –relative wall thickening

*CYP11B2* genotyping showed that there were 31 owners of CC, 77 of TC, and 42 of TT genotypes among 150 examined males. We checked our samples for agreement with Hardy-Weinberg equilibrium using  $\chi^2$  test and found that the differences between actual and theoretically calculated distributions were not significant ( $\chi^2=2.07$ ;  $p=0.35$ ). The distribution of *CYP11B2* polymorphism in the groups of our study is shown in the **Table 2**. It was shown that all groups did not differ significantly, although there were a slight increase of the TC and a decrease of the CC genotype prevalence in hypertensive patients with LVH.

There were no significant differences associated with a *CYP11B2* polymorphism in normotensive men, although in both groups of hypertensive males (with and without LVH), EDD was significantly higher in

allele C carriers (CC and TC genotypes). At the same time, the posterior wall and interventricular septum seemed to be thicker in carriers of T alleles. This trend was statistically significant in hypertensive patients with LVH (Group 3). This explains why there were no differences in LVMI within both groups of hypertensive males, but there were significant increase in RWT with the increase of T allele numbers (**Table 3**).

High echo-reflectivity is a possible marker of disorders in an intrinsic architecture of myocardium. In theory, an increase in high-density elements of myocardium, such as collagen fibers, leads to the increase of ultrasound reflections that could be detected by the device capable to detect the difference in power of waves moving forward and backward, thus, it was so-called backscatter. For more accurate estimation of

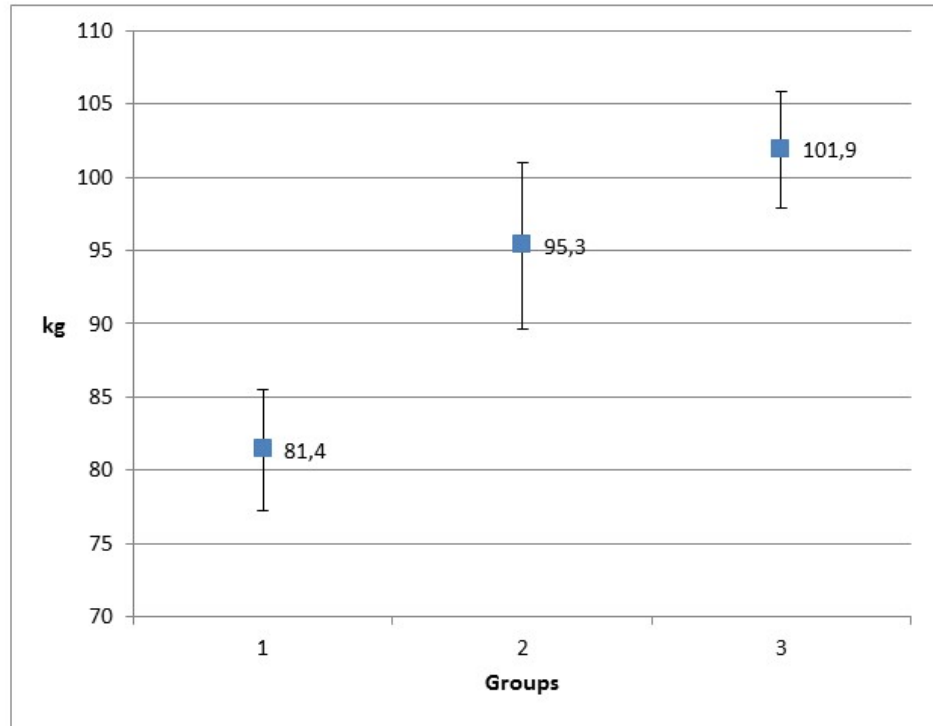


Figure 2: Means of body mass and 95% CI in groups of patients.

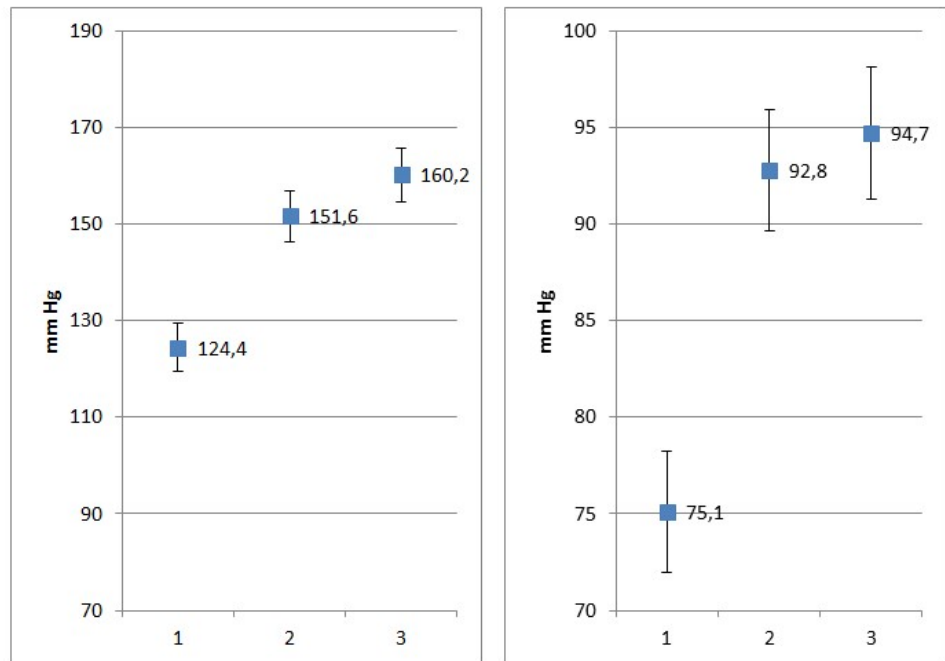


Figure 3: The average values and 95% CI of SBP (left panel) and DBP (right panel) in groups of patients.

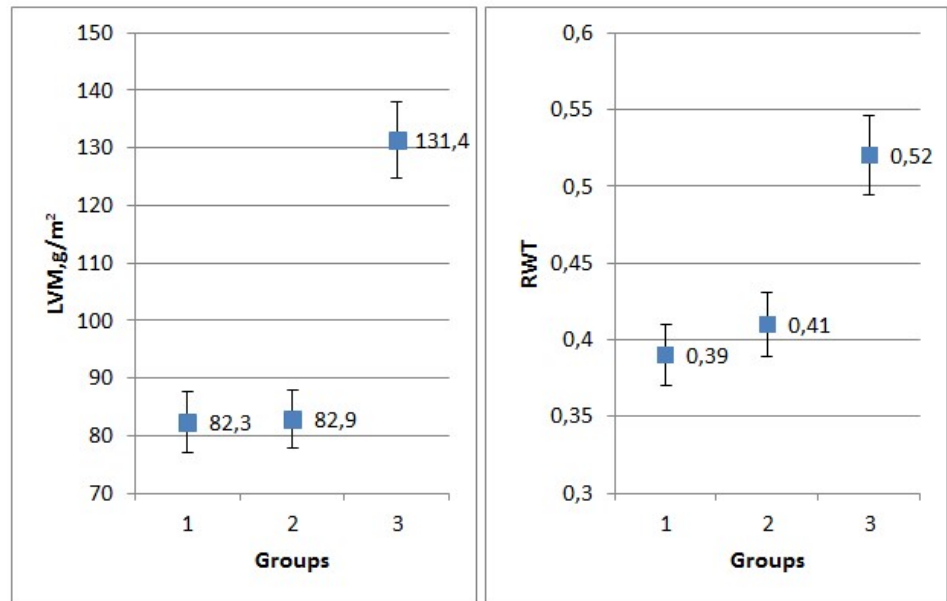


Figure 4: Indexed LVM (left panel) and RWT (right panel) with 95% CI.

Table 2: The distribution of CYP11B2 polymorphism in normotensive, hypertensive, and hypertensive with LV hypertrophy patients

Groups	CC	TC	TT	$\chi^2$	p
NT (n=50)	12 (0.24)	24 (0.48)	14(0.28)		
EH (n=58)	12(0.20)	29 (0.50)	17(0.30)	0.17	0.91
EH+LVH (n=42)	7(0.17)	24(0.57)	11(0.26)	0.98	0.61

$\chi^2$  - the significance of differences of EH and EH+LVH groups comparing with NT group

backscatter, two or more myocardium regions were used for averaging of the means. Also, myocardium backscatter is usually compared with backscatter of the toughest heart structures of the same patient such as pericardium (calibration of backscatter). This method is known as an integrated calibrated backscatter.

Another way of reflected signal estimation is a processing of grayscale images of myocardium by the external software, such as ImageJ, in the offline mode. Since the greyscale has 256 standard tint gradations represented by a certain number of pixels, the general image information can be represented as a column (frequency) graph, which displays the number of pixels for each tint. The most useful parameter of echo-reflectivity analysis is broadband (BB), which indicates the width of the grayscale spectrum. As with the integrated calibrated backscatter, to obtain more accurate meanings, a minimum of 2 regions of interest

(ROI) were chosen: one in the middle of the interventricular septum, and the other — in the posterior wall of left ventricle. The means of BB could be obtained as an average of these two values. Echo-reflectivity was estimated in all patients in the above-mentioned manner.

It was found that the mean range of spectrum in hypertensive males with LVH (Group3) was significantly wider ( $p < 0.001$ ) than in normotensives and hypertensives without LVH (Figure 5).

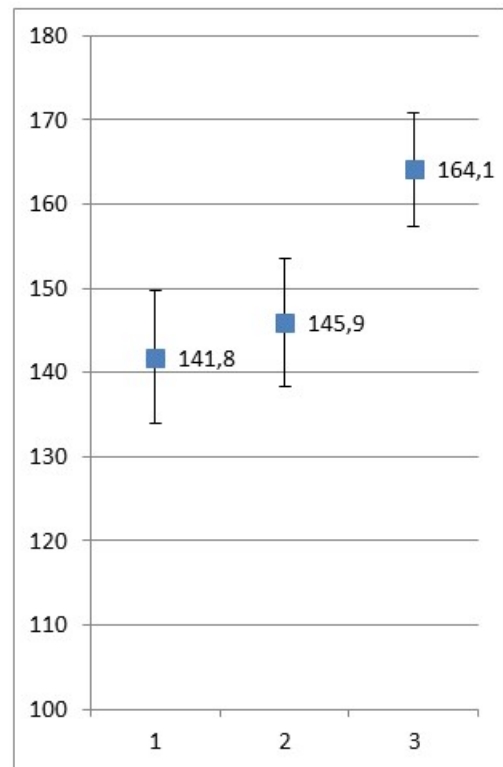
## DISCUSSION

According to current knowledge, hypertensive cardiac remodeling is characterized not only by the increase of the muscle fiber thickness and myocardial mass, but also by the changes in micro-circulation with the extension of relative coronary insufficiency and myocardial fibrosis.

**Table 3: Results of echocardiographic measurements in groups 1, 2, and 3 of patients depending on CYP11B2 polymorphism**

	Group 1				Group 2				Group 3			
	CC (n=12)	TC (n=24)	TT (n=14)	p	CC (n=12)	TC (n=29)	TT (n=17)	p	CC (n=7)	TC (n=24)	TT (n=11)	p
EDD	50.7 (4.4)	48.0 (4.1)	47.6 (5.4)	0.19	52.4 (5.6)	47.8 (4.4)	48.6 (4.8)	0.04	56.2 (6.6)	51.4 (5.0)	50.5 (2.6)	0.01
ESD	34.3 (4.7)	32.4 (3.7)	31.9 (3.9)	0.27	35.5 (4.7)	32.3 (4.2)	33.5 (5.5)	0.19	36.8 (5.3)	34.5 (4.9)	31.9 (3.9)	0.04
PWE	9.1 (1.7)	9.6 (1.2)	9.3 (1.1)	0.50	9.4 (0.7)	10.0 (1.7)	10.0 (1.4)	0.48	11.5 (0.9)	13.2 (1.9)	12.8 (1.4)	0.04
IVSE	9.2 (1.1)	9.2 (1.4)	9.2 (1.8)	0.90	8.9 (1.3)	10.0 (1.6)	9.9 (1.6)	0.12	12.0 (1.7)	13.9 (2.4)	14.6 (2.2)	0.02
LVM	86.1 (13.3)	83.1 (16.4)	77.4 (18.4)	0.37	86.3 (12.1)	81.7 (14.7)	82.6 (16.6)	0.71	121.7 (12.1)	133.3 (25.7)	134.5 (21.3)	0.36
RWI	0.36 (0.07)	0.40 (0.05)	0.39 (0.05)	0.29	0.35 (0.04)	0.43 (0.48)	0.42 (0.08)	0.06	0.43 (0.08)	0.53 (0.12)	0.54 (0.06)	0.02

**Note:** Group 1 – patients with normal pressure; Group 2 – patients with EH without LV changes; Group 3 – patients with EH and LVH; p – significance of differences within genotypes by Kruskal-Wallis ANOVA



**Figure 5: Means and 95% CI for BB in groups 1, 2 and 3.**

There is growing evidence that these factors may be genetically determined, thus, it is likely that the aldosterone synthase gene is one of the possible candidate genes. The data on *CYP11B2* polymorphism prevalence in our study was similar to the studies completed in Ukraine and other countries, where the Caucasian populations such as Germany, Finland, Czech Republic, USA, and Uzbekistan were tested<sup>3-7</sup>. But they were different from orient countries (Japan, China, and Tibet)<sup>8-11</sup> by the prevalence of CC genotype. The associations of larger EDD with the presence of C allele and larger PWD with TT presence were described earlier in a meta-analysis of Wang L. *et al.*<sup>11</sup>. Therefore, our data are in agreement with modern views in this field. The only exception is the increase IVS thickness with T presence, which is in controversy with the abovementioned papers.

Our findings suggest that males with hypertension and LVH differ from patients without LVH, not only by LVMI, but also by the density of myocardium, although they have the same levels of OBP and anthropometric data. Also, the association of *CYP11B2* polymorphism with LV structure remodeling was found in this group of patients but not normotensives and hypertensives without LVH. This remodeling includes significantly larger LVMI and accumulation of fibrotic tissue.

The present study has the limitations of age, gender and number of patients. It is unclear if there are any similar associations of LV structural remodeling with *CYP11B2* polymorphism in other patient groups.

## CONCLUSIONS

The received data allowed the assumption that hypertensive patients with LVH are likely a distinct cluster of patients with their own genetic predisposition to the formation of hypertensive heart disease traits.

## ABBREVIATIONS

**DBP:** Diastolic blood pressure  
**DM:** Diabetes mellitus  
**ECG:** Echocardiography  
**EDD:** End diastolic dimension  
**EF:** Ejection fraction  
**EH:** Essential hypertension  
**ESC:** European society of cardiology  
**ESD:** End systolic dimension  
**LVH:** Left ventricular hypertrophy  
**LVMI:** Left ventricular mass  
**LVMI:** Indexed left ventricular mass  
**OBP:** Office blood pressure  
**PCR:** Polymerase chain reaction  
**ROI:** Region of interest  
**RWT:** Relative wall thickness  
**SBP:** Systolic blood pressure

## COMPETING INTERESTS

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTIONS

**Lozinska M.:** Data collection, statistical processing, literature review, discussion and conclusions final presentation. **Zhebel V.:** General conception and design, final improvement. **Lozinsky S.:** Study coordination, preliminary data analysis, took part in discussion and conclusions generation. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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