

The inappropriateness of left ventricular mass and echoreflectivity in males with essential hypertension and different *CYP11B2* gene polymorphisms

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Abstract

Background. The *CYP11B2* gene as the main controller of aldosterone plasma activity is likely to be responsible as for the BP level as for the expression of different traits of hypertensive cardiac remodeling such as increased left ventricular mass and myocardial fibrosis. The main objective of our study was to define the differences in myocardial remodeling depending on *CYP11B2* gene polymorphism. It was shown that some special techniques of echocardiography such as the assessment of inappropriate left ventricular mass and myocardial echoreflectivity proved additional information in patients with hypertensive heart disease. That's why these techniques were used for a more precise assessment of hypertensive cardiac remodeling.

Material and methods. Our study involved 150 males aged 45–60 years. They were divided into three groups: Group 1–50 patients with normal BP without any echocardiographic abnormalities, Group 2–52 patients with essential hypertension without left ventricular hypertrophy, and Group 3–48 patients with essential hypertension and left ventricular hypertrophy.

Results. It was found that in patients with inappropriate LVM the prevalence of CC genotype was almost twice higher than among those with appropriate LVM. On the other hand, hypertensive patients with CC genotype and LVH demonstrated higher echoreflectivity parameters.

Conclusions. We assume that CC polymorphism of *CYP11B2* may be an indicator of more expressed signs of hypertensive cardiac remodeling, in particular myocardial hypertrophy and myocardial fibrosis, in males with essential hypertension.

Key words: cytochrome P-450 *CYP11B2*; polymorphism; single nucleotide; left ventricular remodeling; essential hypertension

Arterial Hypertens. 2019, vol. 23, no. 4, pages: 240–247
DOI: 10.5603/AH.a2019.0020

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Introduction

The *CYP11B2* gene as the main controller of aldosterone plasma activity is likely to be responsible for the BP level and expression of different traits of hypertensive cardiac remodeling. The main objective of our study was to define the differences in myocardial remodeling depending on *CYP11B2* gene polymorphism.

The increased left ventricular mass or left ventricular hypertrophy (LVH) remains the most often mentioned trait of hypertensive remodeling. A huge number of clinical trials confirmed the negative influence of LVH on the prognosis of essential hypertension. There is also some evidence that the left ventricular mass inappropriate to the BP level has additional negative predictive meaning [1–3].

Myocardial fibrosis is also an essential trait of hypertensive heart disease, but is more difficult to reveal compared with LVH. Changes in myocardial density that are following fibrosis could be detected by analysis of echoreflectivity. This technique is one of the possible approaches for the quantitative estimation of myocardial fibrosis. Echoreflectivity has been shown to have a strong correlation with collagen volume fraction [4]. Therefore, the echoreflectivity analysis was chosen for the assessment of myocardial fibrosis.

Material and methods

The study involved men who were the ambulatory patients of Vinnytsia Regional Dispensary. The sample size of the patients with EH was calculated using Altman nomogram. The statistical power β was set as 0.85 and p as 0.05. The minimal difference in LVM that needed to be determined in groups with different genotypes was accepted as $10 \text{ g/m}^{2.7}$ with the known mean SD approximately equal to $16 \text{ g/m}^{2.7}$. Based on such parameters, the minimal sample size was determined as 90 persons.

The protocol of the study was approved by the local ethics committee. The informed consent was mandatory for all participants. Between 2016 and 2017, 654 patients with EH aged 45–60 years were screened to identify 100 patients with EH. Among them, 58 had no irreversible target organs damages and 42 had left ventricular hypertrophy (LVH). Mean duration of the disease in patients with hypertension was 12.7 years with no substantial difference depending on LVH presence. Three BP measurements were performed at the first visit with 1–2 min interval between them. Additional measurements were made if the first two readings differed by $> 10 \text{ mm Hg}$.

The average of the last two BP readings was recorded. BP was measured in both arms at the first visit to detect possible between-arm differences. Then the arm with the higher value used as the reference. Diagnosis of hypertension was established according to the ESC recommendations (2018).

The control group consisted of 50 normotensive males of the same age without any pathological changes detected by echocardiography (ECG). The criteria for exclusion from the study were as follows:

- congenital or acquired heart defects;
- systemic connective tissue diseases;
- endocrine diseases;
- chronic kidney disease;
- secondary arterial hypertension;
- myocardial disease and heart failure, not associated with arterial hypertension;
- pulmonary arterial hypertension;
- hemodynamically significant arrhythmias (atrial fibrillation or flutter, AV block of 2nd and 3rd degree, high-grade premature beats);
- unsatisfactory ultrasound visualization of the heart;
- type 1 DM or uncontrolled type 2 DM;
- unstable or variant angina at the day of inclusion.

The patients included in the study did not receive antihypertensive therapy (treatment-naïve patients or those with a temporary pause in treatment for more than 1 month). Each participant underwent the following assessments: office blood pressure measurement, echocardiography with echoreflectivity analysis, and determination of the C-344T 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 height^{2.7} (LVMI), ejection fraction (EF), and relative wall thickness (RWT) were obtained by echocardiography. Echocardiography was performed using “Sigma 5000” equipment (Kontron Medical, France). Based on echocardiography measurements the appropriateness of the left ventricular mass to the level of arterial pressure was determined by the formula proposed by De Simone et al. [1]:

$$LVMP = 55.37 + 6.64 \times \text{height (m)}^{2.7} + 0.64SW - 18.07 \times \text{gender (g)}$$

where LVMP is a predicted LVM, SW is a stroke work calculated by the formula: $SW = SBP \times SV \times 0.0144$, and the gender is 1 for men and 2 for women. In the case of exceeding the predicted values, the mass of the myocardium was considered as inappropriate.

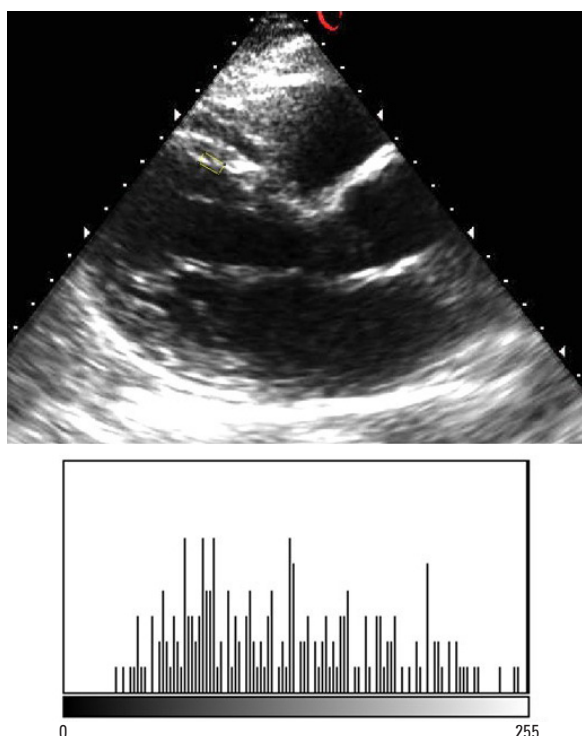


Figure 1. Analysis of echoreflectivity. The left panel shows an left ventricle (LV) image with the region of interest (ROI) marked by a yellow rectangle. The right panel shows the grayscale spectrum of the ROI

Simultaneously with the standard ECG, we located 2 representative areas 20×10 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 processing, the broadband (BB) of the black and white spectrum and mean color scale value (MCSV) of chosen regions was calculated by “Image J” software in the offline mode. Figure 1 demonstrates an example of such processing of the image. A more detailed description of this technique was published in [5].

According to majority of the sources, these parameters are the most reliable echoreflectivity markers of myocardial fibrosis. In an experimental study, its correlation with collagen volume fraction measured by biopsy was as high as 0.72 ($p = 0.03$) [4].

The polymorphism of the *CYP11B2* gene was determined in venous blood samples of all participants by the PCR method.

Genomic DNA was extracted using a set of reagents for the extraction of genomic DNA from a blood sample (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 μ l and consisted of:

- specific oligonucleotide primers: 5'-CAG GAG GAG ACC CCA TGT GAC-3'; 5'-CCT CCA CCC TGT TCA GCC C-3';
- 2.5 μ 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 μ l of mineral oil was poured into the test tubes.

The amplification was performed on the “Tertsik” amplifier (LLC “DNA-Technology”, Russia) using the amplification program, which includes the initial denaturation of 35 cycles at a temperature 94°C during 5 minutes.

For the identification of the alleles, we carried out a restrictive analysis of the amplicons using endonuclease restriction HaeIII (SibEnzyme, Russia) at 37°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-H₃BO₃ and 2 mM EDTA, pH 8.0) for 1 hour at 3–4 V per 1 cm of gel. PbR322/AluI was used as a DNA marker of molecular weight. Gels were stained with ethidium bromide, followed by visualization of results with UV-light.

Results and discussion

A total of 50 men with normal blood pressure and 100 patients with AH were examined. Among the latter, 58 participants had hypertension without any damages of the target organs and, respectively, had normal LVM. Other 42 patients had hypertension with the ECG signs of LV hypertrophy, established based on the values of LVM index ≥ 50 g/m^{2.7}. The results of anthropometry and standard echocardiography are presented below (Tab. I).

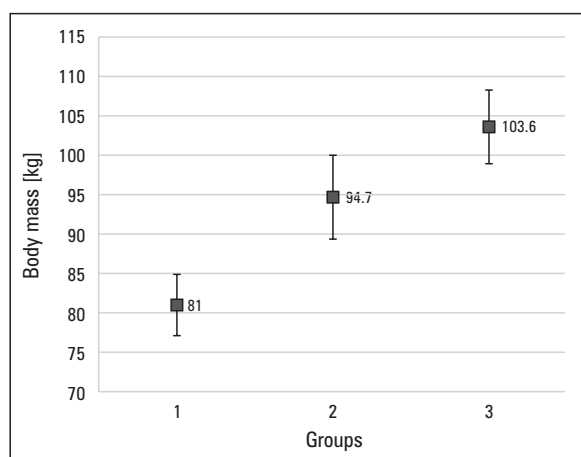
The groups did not differ in age or height, but there were significant between-group differences in body mass ($p < 0.001$ by ANOVA test): the lowest values were observed in Group 1, and the highest — in Group 3 (Fig. 2). The same trend was seen for mean SBP and DBP values ($p < 0.001$) (Fig. 3).

In contrast, changes in LVM differed dramatically: the indexed mean values of LVM were almost the same in Group 1 and Group 2, while in Group 3 they were substantially higher (Fig. 3). Similar pattern of RWT changes was observed.

Table 1. Anthropometry and echocardiography measurements in Groups 1, 2 and 3

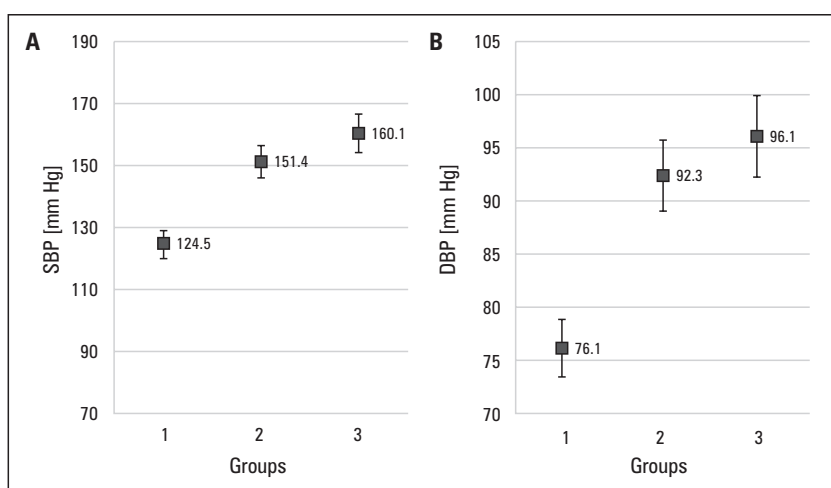
	NT (Group 1) M (SD) n = 50	EH (Group 2) M (SD) n = 58	EH + LVH (Group 3) M (SD) n = 42
Mean age (y.)	49.4 (5.0)	49.6 (5.8)	49.2 (5.8)
Height [cm]	175.1 (11.4)	176.5 (6.8)	178.4 (5.5)
Weight [kg]	81.0 (14.0)	94.7 (19.2)	103.6 (16.8)
SBP [mm Hg]	124.5 (16.4)	151.4 (18.2)	160.1 (23.0)
DBP [mm Hg]	76.1 (9.9)	92.3 (12.0)	96.1 (13.8)
EDD [mm]	48.6 (4.6)	48.9 (4.9)	52.1 (5.2)
ESD [mm]	32.7 (4.1)	33.3 (4.7)	34.3 (4.9)
EF (%)	60.5 (7.8)	59.8 (7.7)	61.9 (9.8)
LVM [g/m ²]	36.4 (14.5)	37.3 (7.0)	60.3 (12.0)
RWT	0.39 (0.06)	0.41 (0.09)	0.52 (0.11)

NT — normotension; EH — essential hypertension; EH + LVH — essential hypertension and left ventricular hypertrophy; SBP — systolic blood pressure; DBP — diastolic blood pressure; EDD — end-diastolic dimension; ESD — end systolic dimension; EF — ejection fraction; LVM — left ventricular mass; RWT — relative wall thickening

**Figure 2.** Means of body mass and 95% confidence interval (CI) in three groups of patients

We revealed that mean values of LVMP calculated using the formula (1) increased relatively evenly, while mean actual LVMI values changed unevenly and the differences between Group 3 and Group 2 were much more prominent and significant compared with the differences between Group 2 and Group 1 group (Fig. 4).

We noticed that despite very similar mean BP values in Group 2 and Group 3 (Fig. 3), Group 3 had the substantially greater LVM (Fig. 4). Moreover, the difference between actual LVM and LVMP in Group 2 was not significant [172.9 (34.9) *vs.* 162.4 (25.7) g; $p > 0.05$] while in Group 3 LVM was substantially higher than LVMP [287.4 (53.9) *vs.* 189.4 (37.8) g, $p < 0.001$ by the Wilcoxon paired test]. We believe that this is probable evidence of an intrinsic

**Figure 3.** The average values and 95% confidential interval (CI) of systolic blood pressure (SBP) (A) and diastolic blood pressure (DBP) (B) in three groups of patients

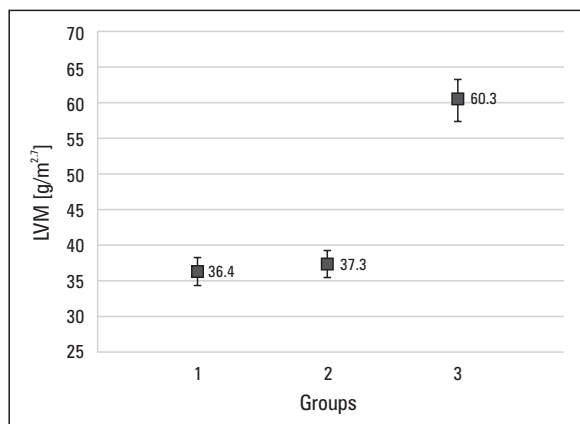


Figure 4. Average indexed left ventricular mass (LVM) and 95% confidence interval (CI) in three groups of patients

(genetic?) difference between patients with and without LVH. Therefore, some additional markers are required for more accurate prediction of appropriate LVM in these patients.

The distribution of *CYP11B2* polymorphism was almost the same in patients with normal BP and with EH regardless of LVH presence (Tab. II).

However, when all the patients were re-divided depending on predicted values of LVM, the differences in distribution become evident (Fig. 6).

As it can be seen in the diagrams, the prevalence of CC polymorphism of *CYP11B2* gene in males with inappropriate LVM was almost twice higher than among those with appropriate LVM. These differences were statistically significant by χ^2 criterion ($p = 0.015$).

Increasing echoreflectivity may be a marker of disorders the intrinsic architecture of myocardium. In theory, an increase in high-density elements of myocardium such as collagen fibers leads to the increase in ultrasound waves reflections that could be detected by the device capable to detect the difference in power of waves moving forward and backward, so-called backscatter. For more accurate estimation of backscatter, 2 or more myocardium regions were used for averaging of the results. In addition, myocardium backscatter is often compared with backscatter of the toughest heart structures of the same patient such as pericardium (calibration of backscatter). That's why this method is known as integrated calibrated backscatter.

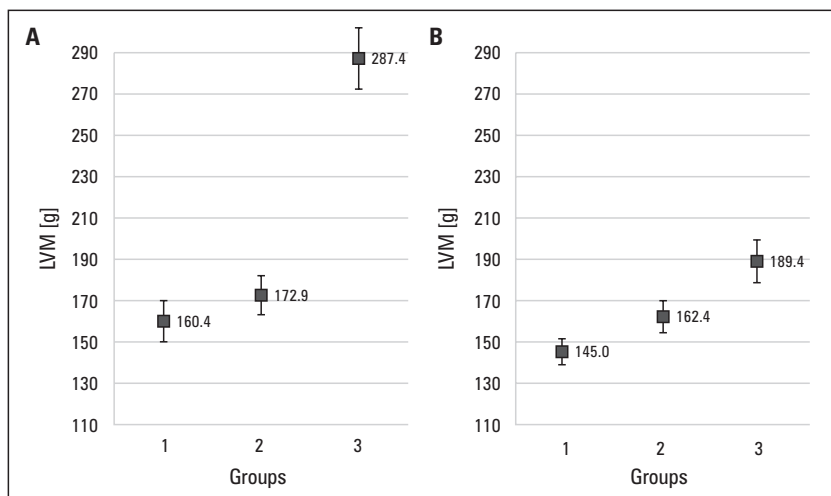


Figure 5. Means and 95% confidence interval (CI) for actual (A) and predicted (B) indexed left ventricular mass (LVM)

Table II. The distribution of *CYP11B2* polymorphism in normotensive patients, hypertensive patients and hypertensive patients with left ventricular hypertrophy

Groups	CC	TC	TT	χ^2	p
NT (n = 50)	12	24	14		
EH (n = 58)	12	29	17	0.23	0.88
EH + LVH (n = 42)	7	24	11	0.77	0.68

χ^2 — the significance of differences when compared EH and EH + LVH groups with NT group; NT — normotension; EH — essential hypertension; EH + LVH — essential hypertension and left ventricular hypertrophy

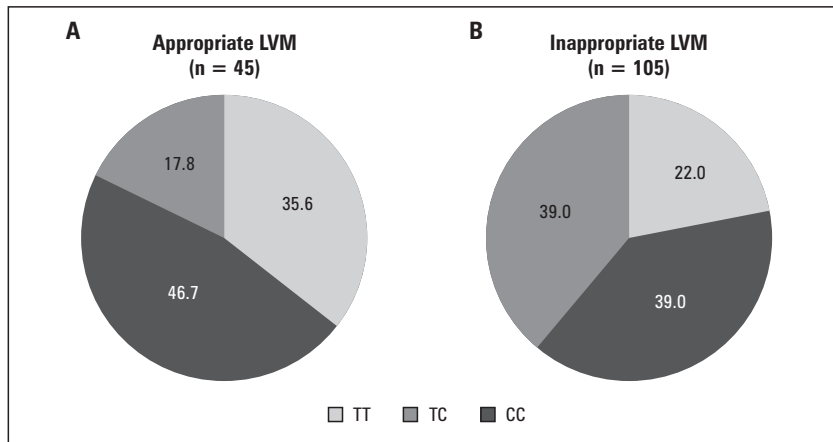


Figure 6. Frequencies (%) of *CYP11B2* gene polymorphisms in patients with appropriate (A) and inappropriate (B) left ventricular mass (LVM)

Table III. Mean echorefectivity parameters in Groups 1, 2 and 3. Data are presented as means (SD)

Parameter	Group 1	Group 2	Group 3
MCSV	62.3 (28.5)	59.9 (32.4)	74.5 (36.5)
BB	105.3 (51.5)	109.0 (37.0)	135.4 (22.8)

MCSV — mean color scale value; BB — broadband of the black and white spectrum

Another way of reflected signal estimation is processing of grayscale images of myocardium by the external software in the offline mode such as “Image J”. Since the gray scale has 256 standard 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 parameters are the mean color scale value (MCSV) and broadband (BB). As with the integrated calibrated backscattering analysis, in order to obtain more objective information, a minimum of 2 regions of interest (ROI) were chosen: one is in the middle of the interventricular septum, and the other — in the posterior wall of left ventricle. The values of MCSV and/or BB could be calculated as a mean of these two readings.

Echorefectivity was estimated in all of the patients in the above-mentioned manner. Obtained results are shown below (Tab. III).

In patients with EH and LVH (Group 3) the values of MCSV were higher than in normotensive and hypertensive patients without LVH, although the statistical significance of the differences was low ($p = 0.066$). The range of the grayscale spectrum (BB) in patients with LVH was significantly higher ($p < 0.001$) than in other study groups (Fig. 7) and looked similar to the trend of LVM (Fig. 4). This fact

supports our suggestion about intrinsic differences between patients with and without LVH.

Echorefectivity was also analyzed in groups created on the basis of the predicted values of LVM and having appropriate or inappropriate LVM. As it was mentioned earlier, these groups consisted of 45 and 105 men respectively. The mean values of MCSV and BB were higher in males with inappropriate LVM than in those with appropriate LVM (Fig. 8).

At the present time, we know that hypertensive cardiac remodeling includes the increase in the thickness of muscle fibers and myocardial mass but also changes in microcirculation with the extension of relative coronary insufficiency and myocardial fibrosis. The detection of exceed in myocardial mass is a relatively simple diagnostic task. However, myocardial fibrosis and coronary failure often remain unnoticed, which leads to the underestimation of their role in hypertensive heart disease. On the other hand, the old question of whether LVH is always a pathological process in persistent hypertension, and where the borderline passes between compensatory and pathological hypertrophy, still remains open. Our recent results allow assuming that the determination of the appropriateness of the left ventricular myocardial mass to the level of blood pressure could be useful not only concern-

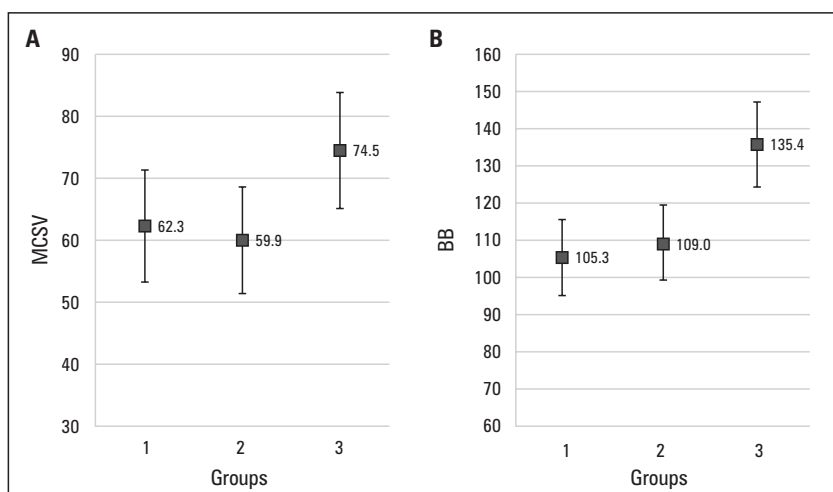


Figure 7. Means and 95% confidence interval (CI) for mean color scale (MCSV) (A) and broadband of the black and white spectrum (BB) (B) in three groups of patients

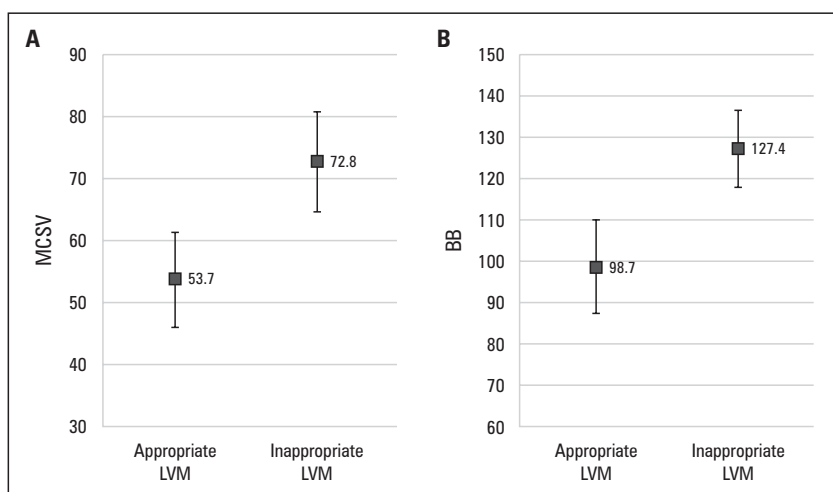


Figure 8. Means and 95% confidence interval (CI) for mean color scale (MCSV) (A) and broadband of the black and white spectrum (BB) (B) in groups with appropriate and inappropriate left ventricular mass (LVM)

ing the severity of hypertrophy but also concerning echorefectivity that is considered a marker of myocardial fibrosis. In this case, a greater prevalence of the inappropriate LVM in patients with such a structure of the gene *CYP11B2* could indicate on a genetic predisposition for more expressive manifestations of hypertensive heart disease at the same level of blood pressure. Therefore, we can assume that the CC polymorphism of the *CYP11B2* gene could serve as a marker of more expressed remodeling, including myocardial fibrosis and myocardial hypertrophy.

The method used to determine the appropriate mass of the left ventricle in this study showed almost linear growth of the calculated values in the

study groups, while the real values had a character that resembles an exponential curve. Therefore, despite the proven additional predictive value of the appropriate LV mass, this method requires adaptation to real practice. Another conclusion is that the mass of left ventricle does not depend directly on the level of BP. Consequently, other factors, responsible for the hypertensive cardiac remodeling, besides the hemodynamic load, may be hypothesized. There is growing evidence that at least some of these factors may be of genetic origin and aldosterone synthase gene could be among them. At least, the data obtained are not in controversy with such a hypothesis, but to some extent they support it.

Therefore, the next step may be to compare the mass and myocardial reflectivity in patients with different *CYP11B2* polymorphisms, standardized for other parameters and risk factors.

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