J Med Sci 2024;44 (1):18-25
DOI: 10.4103/jmedsci.jmedsci_66_23

ORIGINAL ARTICLE

Novel Genetics and Humoral Prognostic Markers of Left Ventricle Hypertrophy in Hypertensive Patients

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Background: Left ventricular hypertrophy (LVH) is not only complications or the damaged appearance of the target organ of patients with essential arterial hypertension (EAH), but at the same time, it is also a prognostic factor. **Aim:** The aim is to evaluate the echocardiographic (Echo-CG) changes in patients with EAH depending on genes AGT (rs4762), GNB3 (rs5443), and some humoral markers. **Methods:** A total of 100 EAH patients were recruited for this study. AGT (rs4762) and GNB3 (rs5443) genotyping were performed by Real-Time PCR. All recruited individuals were tested for serum levels of ionized calcium, parathyroid hormone, and 25-hydroxyvitamin D. LVH was assessed using Echo-CG. **Results:** T-allele of the AGT (rs4762) and GNB3 (rs5443) genes are associated with myocardial structure changes in hypertensive patients: thicker relative wall thickness (RWT), interventricular septum in diastole and higher left ventricular (LV) mass index (LVMI) in women for GNB3 (rs5443) gene. Hypovitaminosis D in EAH patients is accompanied by LV remodeling: larger left atrium size, LV mass (LVM), and LVMI in women, with lower ejection fraction. Hypocalcemia links to the smaller wall thickness of hypertrophied LV myocardium in EAH individuals than in the case of its normal concentration, as well as a lower LVMI and better LV contractile function, which confirms the hypothesis of active involvement of calcium in the process of myocardial remodeling and hypertrophy. **Conclusion:** The polymorphic variants of the AGT (521 C>T) and GNB3 (825 C>T) genes are associated with myocardial structure changes in EAH patients. Hypovitaminosis D in hypertensive individuals is accompanied by LVM and LVMI increase but reliably only in women.

Key words: Echocardiography, left ventricle hypertrophy, arterial hypertension, *AGT* (rs4762), *GNB3* (rs5443) genes polymorphism, Vitamin D, ionized calcium, parathyroid hormone

INTRODUCTION

Myocardial remodeling under renin–angiotensin–aldosterone system (RAAS) activation is caused by a number of factors: structural, metabolic, hemodynamic, neurohumoral, epigenomic, genetic, inflammatory mechanisms of cytokine activation, etc., which affect both directly myocardial cells and indirectly through the extracellular matrix and/or vascular endothelium.[1-7]

The receptors for cardiotrophin-1 (CT-1) are present in many organs and tissues, and its effect has been proven mainly on the heart muscle: promitotic and proliferative activity, induction of myocardial hyperplasia and cardiomyocyte hypertrophy.[8,9] However, cardiomyocytes also secrete the cytokine CT-1 independently into the coronary vein system.[10] According to some studies, the level of CT-1 in plasma correlates with the left ventricular hypertrophy (LVH) size[11] and the development of diastolic dysfunction.[12] A circulus vitiosus is formed when CT-1 production and gp130/LIF expression increase in response to arterial hypertension pressure and/or volume overload, myocardial stretch, and increased myocardial

Received: March 09, 2023; Revised: May 29, 2023; Accepted: June 10, 2023; Published: September 29, 2023
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How to cite this article: Voroniuk K, Sydorchuk L, Repchuk Y, Sydorchuk A, Sydorchuk R, Iftoda O. Novel genetics and humoral prognostic markers of left ventricle hypertrophy in hypertensive patients. J Med Sci 2024;44:18-25.

stiffness. In addition, the CT-1 and gp130/LIF production and activity are regulated by a number of neurohormones and RAAS peptides (norepinephrine, angiotensin II, aldosterone, urocortin, etc.), as well as phosphorus and calcium metabolism.[9,10] It should be noted that the influence of the immune system through cytokine inflammatory mechanisms on the processes of heart and vascular remodeling (through the activation of macrophages, endothelial cells, free oxygen radicals, oxidative stress, and increased ionized Ca^{2+}) has been studied by some researchers.[11-13] At the same time, oxidative stress through the hydrogen sulfide and superoxide anion systems can increase the expression of inflammatory response genes and enhance RAAS activity, especially in hypercholesterolemia.[9] The latter prompts discussion about the possible influence of metabolic and genetic factors on RAAS activity and the development of a certain type of LVH.[14-17]

Therefore, we aimed to study the echocardiographic (Echo-CG) parameters depending on the allelic state of the AGT (521 C>T) and GNB3 (825C>T) genes, blood concentrations of parathyroid hormone (PTH), Vitamin D and ionized calcium.

MATERIALS AND METHODS

Study design and patients

The study was conducted in full compliance with the main ethical principles of the European Convention on Human Rights and Biomedicine, according to the standards of the Helsinki Declaration, GLP and GCP, EUC directive #609 and other EU and international legislations on bioethics and was approved by The Biomedical Ethics Commission of the BSMU of the Ministry of Health of Ukraine (Chernivtsi), Approval number - 3 Approval date – 01.03.2023. Each participant signed a consent form to participate in the study. The Research is defined as a prospective, cohort, case–control study.

Diagnosis inclusion/exclusion criteria

Hypertension was defined according to the European Societies of Hypertension and Cardiology (ESH/ESC) recommendations: office systolic BP (SBP) values ≥140 mmHg and/or diastolic BP (DBP) values ≥90 mmHg at least for three measurements during a month.[10,11]

The study enrolled essential arterial hypertension (EAH) patients with hypertensive-mediated organ damage (HMOD) estimated according to ESH/ESC recommendations (ESH/ESC 2018, 2021):[10,11] target-organs damage – 2nd stage (asymptomatic EAH), moderate-high-very high cardiovascular risk (CVR), from the 1st through to the 3rd grade of BP elevation.

Exclusion criteria were presented in our former publications:[16,18,19] EAH patients with complicated/symptomatic HMOD (coronary heart disease, heart attack, stroke, heart failure, aneurysm, chronic kidney diseases, thickened, narrowed or torn blood vessels in the eyes, carotid arteries intima-media thickness enlargement, peripheral artery disease, etc.); secondary EAH; malignant or uncontrolled EAH; diabetes mellitus type I, sub- and decompensated diabetes mellitus type 2 (with diabetic target-organ damage); sub- and decompensated liver diseases; bronchial asthma, chronic obstructive pulmonary disease of III–IV stage with C or D risk value (GOLD 2019); exacerbated infectious diseases or during unstable remission of any location, including systemic immune system diseases; severe dementia; psychological/psychiatric disorders/diseases; malignancies of any location; multiple organ failure; use of oral corticosteroids or contraceptives; pregnancy or lactation.

One hundred patients were selected for further examination after the screening of matching inclusion and exclusion criteria: 79% of women, 21% of men, mean age 59.86 ± 6.22 years. The control group included 60 practically healthy individuals who were not relatives of the patients, without reliable differences in age (49.13 ± 6.28) and gender distribution (63% - women, 37% - men) with the study group.

Laboratory and clinical data collection

All recruited patients were observed by general physicians, cardiologists and underwent a complex of basic clinical examinations: clinical anamnesis recording, anthropometric parameters, body mass index (BMI, kg/m^2), complete blood count, total cholesterol level, low-/high-density level cholesterol (LDL-, HDL-C), serum uric acid, office SBP, DBP and heart rate measurement, ECG in 12 leads, EchoCG, kidneys' ultrasound examination and Daily Holter BP monitoring in undetermined conditions according to Ukrainian standards (2019) and European recommendations ESC/ESH (2018, 2021).

All patients and healthy individuals were tested for serum levels of ionized calcium (Ca^{2+}) (potentiometry, "SINNOWA", China), PTH and 25-hydroxyvitamin D (Vitamin D) (immune luminescent test "MAGLUMI", "SNIB", China).

Left ventricular hypertrophy patterns

The LVH was estimated using the established ECG criteria: Sokolow–Lyon index and Cornell scoring system.

The transthoracic echocardiography (Echo-CG) in M- and B-modes was utilized to confirm the LVH and the structural and functional myocardium state analysis, including the left ventricular (LV) geometry. The standard linear Echo-CG indicators were measured by Ultrasonography complex "ACCUVIX A30" (Samsung Medison, South Korea). The LV mass (LVM) was calculated according to the Penn Convention. LV mass index (LVMI) was assessed by LVM/body surface area ratio (g/m^2). LVMI cutoff values of Echo-CG LVH

diagnostic criteria were >115 g/m2 for men and >95 g/m2 for women (ESC/ESH, 2018). According to LVMI and LV relative wall thickness (RWT), the following geometric models of LV were identified (ESC/ESH, 2009): normal geometry of LVconcentric remodeling of LVeccentric LVH, concentric LVH.

Genotyping assay

Venous blood was collected in a sterile vacutainer, and stabilized by K2-EDTA. DNA was isolated from the whole venous blood lymphocytes' nuclei and purified according to the GeneJET Genomic DNA Purification Kit manufacturer's instructions (Thermo Fisher Scientific, USA). DNA fragments of analyzed genes amplified by Quantitative Real-Time PCR with specific for each gene TaqMan probes and genotyping with TaqMan Genotyping Master Mix on CFX96 Touch™ Real-Time PCR (RT-PCR) Detection System (Bio-Rad Laboratories, Inc., USA). The genotyping protocol was described in our former publications.[12,13] Alleles' discrimination of *AGT* (rs4762) and GNB3 (rs5443) genes polymorphisms was analyzed by licensed CFX96 RT-PCR Detection System Software (Microsoft, USA). The genetic examination was performed for 72 patients and 48 healthy subjects.

Statistical analysis

StatSoft Statistica ® v. 7.0 software was used for statistical analysis (StatSoft Inc., USA). The genotype distribution was assessed by the Pearson test (c^2). To verify the differences between groups, we applied the Students *t*-test (two-tail distribution and equal variances between the two samples), ANOVA, or the Wilcoxon–Mann–Whitney U-test (in case of uneven data distribution according to W–Shapiro–Wilk or Kolmogorov–Smirnov test results). $P < 0.05$ was considered statistically significant.

RESULTS

In our research women dominated over men 2.72 times in general with no reliable differences in mean age and gender distribution depending on groups [Table 1]. Twenty EAH patients have compensated Diabetes Mellitus type 2, 15% were smokers, 74 patients have burdened family history by cardiovascular disease, 95% – overweight or obese.

The association of the *AGT* gene (rs4762) allelic state with Echo-CG data showed that EAH patients carrying the mutational *T*-allele had higher posterior wall thickness (PWT), increased IVSd and RWT – by 2.56% ($P_{CC} = 0.009$), 2.52% ($P_{CC} = 0.017$) and 4.54% ($P_{CC} < 0.001$) than those with the *CC* genotype, respectively [Table 2]. In addition, there was a clear dependence of LVMI on the *AGT* gene polymorphism (rs4762)

Table 1: Some demographic data and anthropometric parameters of the examined population

Parameters	Control (*n*=60), *n* (%)	Patients (*n*=100), *n* (%)
Mean age (years)	49.13±6.28	59.86±6.22
Sex		
Women	38 (63.33)	79 (79.0)
Men	22 (36.67)	21 (21.0)
Smokers	6 (10.0)	15 (15.0)
DM 2	0	20 (20.0)
Family history of CVD	27 (45.0)	74 (74.0)
WHR		
Women		
↑ (>0.88)	11 (18.33)	70 (70.0)
N (≤0.88)	27 (45.0)	9 (9.0)
Men		
↑ (>0.95)	15 (25.0)	14 (14.0)
N (≤0.95)	7 (11.67)	7 (7.0)
BMI (kg/m^2)		
Women		
↑ (>25)	25 (41.67)	75 (75.0)
N (≤24.9)	13 (21.67)	4 (4.0)
Men		
↑ (>25)	20 (33.33)	20 (20.0)
N (≤24.9)	2 (3.33)	1 (1.0)

DM2=Diabetes mellitus type 2; CVD=Cardiovascular disease; WHR=Waist-to-hip ratio; BMI=Body mass index

in the control group, regardless of gender, where the index varied within normal limits with higher values in *T*-allele carriers: in women – by 16.88% ($P_{CC} = 0.018$), in men – by 25.97% ($P_{CC} = 0.016$).

The Echo-CG parameters depending on the allelic state of the *GNB3* gene (rs5443) are given in Table 3. Mutational *T*-allele carriers of the GNB3 gene (rs5443) showed a larger aortic diameter (Ao) by 12.88% ($p_{CC} < 0.001$), IVSd and LVMI in women – by 4.24% ($p_{CC} = 0.05$) and 9.85% ($p_{CC} = 0.045$), respectively. The allelic dependence of the GNB3 gene (rs5443) with LVMI was established in the control group in women, where the LVMI varied within normal values, and prevailed in *T*-allele carriers over that in *CC*-genotype individuals by 22.11% ($p_{CC} = 0.024$). A similar tendency was not observed in men. In addition, LVMI in women was generally higher than in men, but significantly only in T-allele patients - by 13.87% ($p_W = 0.049$).

We found no significant deviations of Echo-CG parameters depending on the PTH serum concentration, neither in patients nor in the control group [Table 4].

Table 2: Echocardiographic values in hypertensive patients depending on *AGT* (rs4762) polymorphic variants, M±m

Echo-CG values	*AGT* gene genotypes in the control group		*AGT* gene genotypes in the study group	
	Genotypes	Results	*CC*−	*CT* + *TT*−
Ao (cm)	*CC*−	2.60±0.12	3.51±0.15	3.59±0.13
	T-allele	2.95±0.11	*P*<0.001	*P*=0.042
LA (cm)	*CC*−	2.39±0.14	4.11±0.16	4.16±0.14
	T-allele	2.68±0.15	*P*<0.001	*P*<0.001
EF (%)	*CC*−	68.64±0.93	60.11±1.15	60.26±1.59
	T-allele	69.58±0.76	*P*<0.001	*P*<0.001
PWT (cm)	*CC*−	0.73±0.04	1.17±0.02	1.20±0.02
	T-allele	0.77±0.02	*P*<0.001	*P*<0.001
				P_{CC}=0.009
IVSd (cm)	*CC*−	0.76±0.02	1.19±0.025	1.22±0.02
	T-allele	0.78±0.02	*P*<0.001	*P*<0.001
				P_{CC}=0.017
LVM (g)	*CC*−	85.93±7.18	302.0±12.60	304.45±18.44
	T-allele	96.95±8.22	*P*<0.001	*P*<0.001
LVMI (g/m²)				
Women	*CC*−	44.37±2.06	139.88±7.53	153.42±6.62
	T-allele	51.86±2.14	*P*<0.001	*P*<0.001
		P_{CC}=0.018		
Men	*CC*−	47.67±2.25	137.89±4.62	146.13±5.55
	T-allele	60.05±3.98	*P*<0.001	*P*<0.001
		P_{CC}=0.016		
RWT	*CC*−	0.39±0.01	0.44±0.01	0.46±0.01
	T-allele	0.40±0.02	*P*<0.001	*P*<0.001
				P_{CC}<0.001

P=Significance of differences with control group according to the corresponding genotype; P_{cc}=Significance of differences with *CC* genotype carriers in the corresponding group; P_w=Significance of differences with women in the corresponding group. AO=Aorta; LA=Left atrium; EF=Ejection fraction; PWT=Posterior wall thickness; IVSd=Intra-ventricular septum diastole; LVM=Left ventricular mass; LVMI=LVM index; RWT=Relative wall thickness; Echo-CG=Echocardiographic

Some indicators of myocardial structure and intracardiac hemodynamics in EAH patients varied depending on the Vitamin D (25-hydroxycalciferol) blood concentration [Table 5]. The low Vit D levels (<30 ng/ml) are associated with an increased LA size and LVM by 6.39% ($P = 0.048$) and 13.32% ($P = 0.042$) as well, and a lower EF – by 4.55% ($P = 0.036$), respectively, than in individuals with normal 25-hydroxycalciferol concentrations. LVMI in patients did not depend on the total blood Vitamin D metabolite level. However, the gender distribution showed a higher LVMI in women with reduced Vitamin D levels (<30 ng/ml) than with its preserved concentration: 155.45 ± 6.88 g/m² versus 140.11 ± 5.74 g/m² ($P = 0.048$). In men, there was no significant difference in LVMI, taking into account the Vitamin D level.

Table 3: Echocardiographic values in hypertensive patients depending on *GNB3* (rs5443) polymorphic variants, M±m

Echo-CG values	*GNB3* gene genotypes in the control group		*GNB3* gene genotypes in the study group	
	Genotypes	Results	*CC*−	*CT* + *TT*−
Ao (cm)	*CC*−	2.55±0.14	3.26±0.10	3.68±0.17
	CT + *TT*−	2.66±0.15	*P*<0.001	*P*<0.001
				P_{CC}<0.001
LA (cm)	*CC*−	2.39±0.15	4.11±0.13	4.15±0.12
	CT + *TT*−	3.68±0.16	*P*<0.001	*P*<0.001
EF (%)	*CC*−	68.64±1.93	61.14±1.04	60.22±1.47
	CT + *TT*−	69.58±0.53	*P*<0.001	*P*<0.001
PWT (cm)	*CC*−	0.73±0.04	1.17±0.03	1.16±0.03
	CT + *TT*−	0.77±0.03	*P*<0.001	*P*<0.001
IVSd (cm)	*CC*−	0.76±0.02	1.18±0.02	1.23±0.02
	CT + *TT*−	0.78±0.02	*P*<0.001	*P*<0.001
				P_{CC}=0.05
LVM (g)	*CC*−	85.93±6.73	286.47±15.33	301.14±17.02
	CT + *TT*−	96.95±7.74	*P*<0.001	*P*<0.001
LVMI (g/m²)				
Women	*CC*−	41.25±1.98	140.83±5.51	154.70±7.64
	CT + *TT*−	50.37±2.32	*P*<0.001	*P*<0.001
		P_{CC}=0.024		P_{CC}=0.045
Men	*CC*−	47.84±2.31	139.01±5.69	135.85±3.97
		P_W=0.048	*P*<0.001	*P*<0.001
	CT + *TT*−	51.12±4.88		P_W=0.049
RWT	*CC*−	0.38±0.01	0.45±0.02	0.45±0.01
	CT + *TT*−	0.39±0.01	*P*<0.001	*P*<0.001

P=Significance of differences with the control group according to the corresponding genotype; P_{cc}=Significance of differences with *CC* genotype carriers in the corresponding group; P_w=Significance of differences with women in the corresponding group. AO=Aorta; LA=Left atrium; EF=Ejection fraction; PWT=Posterior wall thickness; IVSd=Intra-ventricular septum diastole; LVM=Left ventricular mass; LVMI=LVM index; Echo-CG=Echocardiographic; RWT=Relative wall thickness

90.28% of EAH patients ($n = 65$) had a decrease in total Vitamin D metabolites (<30 ng/ml), almost every third (34.72%, $n = 25$) had an increase in PTH (>65.0 pg/ml), and 15.28% of patients ($n = 11$) had hypocalcemia ($\bar{}Ca^{2+}$ <1.12 mmol/L). It should be noted that the concentration of calcium in the blood, in cells, and in extracellular space is strictly controlled by the body to maintain normal physiological function.[20,21] Even a slight decrease in blood calcium concentration is a signal for the parathyroid glands to synthesize PTH more intensively. In the kidneys, PTH stimulates the conversion of Vitamin D to its active form (1,25-dihydroxyvitamin D/calcitriol), which reduces urinary calcium excretion but increases phosphorus excretion.[22] PTH and 1,25-dihydroxyvitamin D are involved in calcium metabolism and stimulate its release from bones by activating osteoblasts (decreased bone mineralization) in case

Table 4: Echocardiography values depending on the parathormone blood level, M±m

Echo-CG values	PTH concentration, in the control group (pg/mL)		PTH concentration in the study group (pg/mL)	
	PTH level	Results	↑ PTH ≥65	N PTH <65
Ao (cm)	↑ PTH ≥65	2.67±0.14	3.55±0.13	3.51±0.12
	N PTH <65	2.58±0.11	*P*<0.001	*P*<0.001
LA (cm)	↑ PTH ≥65	2.56±0.12	4.15±0.15	4.13±0.11
	N PTH <65	2.62±0.11	*P*<0.001	*P*<0.001
EF (%)	↑ PTH ≥65	69.06±1.06	60.20±1.10	60.64±1.81
	N PTH <65	69.29±0.86	*P*<0.001	*P*<0.001
PWT (cm)	↑ PTH ≥65	0.79±0.03	1.18±0.02	1.17±0.03
	N PTH <65	0.75±0.03	*P*<0.001	*P*<0.001
IVSd (cm)	↑ PTH ≥65	0.79±0.03	1.21±0.02	1.19±0.02
	N PTH <65	0.80±0.02	*P*<0.001	*P*<0.001
LVM (g)	↑ PTH ≥65	89.64±7.89	305.14±15.44	293.36±14.13
	N PTH <65	93.67±6.44	*P*<0.001	*P*<0.001
LVMI (g/m²)	↑ PTH ≥65	45.78±3.84	149.04±6.72	144.45±8.74
	N PTH <65	50.14±2.96	*P*<0.001	*P*<0.001
RWT	↑ PTH ≥65	0.39±0.01	0.45±0.01	0.45±0.02
	N PTH <65	0.39±0.015	*P*<0.001	*P*<0.001

P=Significance of differences with the control group according to the corresponding PTH level; *P*1=Significance of differences with the PTH level ≥65 pg/mL in the corresponding group. PTH=Parathyroid hormone; AO=Aorta; LA=Left atrium; EF=Ejection fraction; PWT=Posterior wall thickness; IVSd=Intra-ventricular septum diastole; LVM=Left ventricular mass; LVMI=LVM index; RWT=Relative wall thickness, Echo-CG=Echocardiographic

of decreased blood Ca^{2+} concentration.[20,21]

In the EAH patients with the decreased ionized Ca^{2+} blood level, we observed a smaller LV wall thickness than with its normal concentration, as well as lower LVMI with better contractile function of the LV by EF: PWT was thinner by 4.25% ($p_1 = 0.048$), IVSd – by 4.13% ($p_1 = 0.049$), RWT - by 6.67% ($p_1 = 0.017$), borderline lower LVMI – by 6.85% ($p_1 = 0.052$), at higher EF – by 5.22% ($p_1 = 0.046$), respectively [Table 6]. The results confirm the hypothesis of the active involvement of calcium in the process of myocardial remodeling and hypertrophy to ensure the activity of both RAAS components, signaling pathways, and cytokine mechanisms of the immune response (CT-1, gp130/LIF), etc.

DISCUSSION

Nowadays, the pathological mechanisms, as well as key triggers and predictors of hypertrophic ventricular remodeling, have not yet been fully determined.[23] In response to pathophysiological stimuli such as hypertension with excessive mechanical load, or ischemia/reperfusion, or both mechanical and neurohumoral triggers, multiple molecular and cellular processes contribute to cardiomyocyte hypertrophy and ventricular remodeling. Cardiomyocytes could be lost through cell death pathways such as necrosis, apoptosis, or excessive autophagy. Moreover, the accumulation of extracellular matrix leads to fibrosis. Apart from that, metabolic disorders such as insulin resistance (IR) and lipotoxicity can occur. Finally, alterations in ion transporting processes and cardiomyocytes structural changes may culminate clinically with arrhythmic phenotype.[23] In this context, HC Hung *et al*. in their study demonstrated a special role of cytokines (the interleukin family), which includes ciliary neurotrophic factor, IL-11, leukemia inhibitory factor (LIF), oncostatin M, and CT-1 in LVH development. The latter interacts with the glycoprotein gp130 and LIF receptors on myocardial cells, activates them, and stimulates JAK/STAT factors of hypertrophy and apoptosis.[24] This hypothesis was confirmed by lifetime endomyocardial biopsy in 10 patients with EAH, LVH and preserved LV function and 14 patients with EAH, LVH and manifest chronic heart failure (CHF) caused by AH.[9,10,25] At the same time, the gp130 signaling pathway was suppressed in EAH patients at the onset of CHF.

In turn, the results of our study confirm the hypothesis of the active involvement of calcium in the process of myocardial remodeling and hypertrophy to ensure the activity of both RAAS components and cytokine mechanisms of the immune response (CT-1, gp130/LIF). Regarding genetic markers, it has been proven that a number of mutated genes encoding sarcomere proteins, actin filaments connecting cardiomyocyte myofibrils (MYH7, MYBPC3, genes encoding the heavy chain of β-myosin and myosin-binding protein C), have a direct etiologic link in patients with hypertrophic cardiomyopathy (HCM) and LVH in other pathologies.[15] We have found that polymorphic variants of the *AGT* (rs4762, 521 C>T) and *GNB3* (rs5443, 825 C>T) genes are associated with alterations in myocardial structure and intracardiac hemodynamics in EAH patients.

Lai *et al*. established that IR and Vitamin D deficiency are independent predictors of LVH and atherosclerotic disease and are associated with increased CVR.[26]

In our study, hypovitaminosis D in hypertensive individuals is accompanied by LVM and LVMI increase but reliably only in women. The functional or absolute Vitamin D deficiency may predispose to the development of LVH by impairing cardiac contractility, increasing myocardial collagen content, altering myosin protein expression, and activating the renin-angiotensin axis[27] Moreover, recent studies found that people with obesity have lower blood levels of Vitamin D than people of healthy weight.[28] In our study, overweight and obese women were dominated over men (almost three times

Table 5: Echocardiography indicators in subjects depending on the level of Vitamin D (25-hydroxycalciferol) in the blood M±m

Echo-CG values	Vitamin D concentration in the control group (ng/mL)		Vitamin D concentration in the study group (ng/mL)	
	Vitamin D level	Results	↓ Vitamin D <30	N Vitamin D ≥30
Ao (cm)	↓ Vitamin D <30	2.64±0.13	3.54±0.13	3.41±0.15
	N Vitamin ≥30	2.58±0.08	*P*<0.001	*P*=0.003
LA (cm)	↓ Vitamin D <30	2.59±0.13	4.16±0.11	3.91±0.06
	N Vitamin ≥30	2.57±0.12	*P*<0.001	*P*<0.001
				P_1=0.048
EF (%)	↓ Vitamin D <30	67.50±0.73	60.23±1.14	63.10±0.70
	N Vitamin ≥30	69.58±0.75	*P*<0.001	*P*=0.003
		P_1=0.041		P_1=0.036
PWT (cm)	↓ Vitamin D <30	0.77±0.03	1.17±0.03	1.17±0.02
	N Vitamin ≥30	0.77±0.02	*P*<0.001	*P*<0.001
IVSd (cm)	↓ Vitamin D <30	0.80±0.02	1.20±0.02	1.19±0.02
	N Vitamin ≥30	0.77±0.02	*P*<0.001	*P*<0.001
LVM (g)	↓ Vitamin D <30	96.25±6.89	300.49±14.09	265.18±9.51
	N Vitamin ≥30	89.16±4.64	*P*<0.001	*P*<0.001
				P_1=0.042
LVMI (g/m²)	↓ Vitamin D <30	50.09±3.80	146.50±7.03	141.39±4.37
	N Vitamin ≥30	46.80±2.51	*P*<0.001	*P*<0.001
RWT	↓ Vitamin D <30	0.39±0.01	0.45±0.01	0.47±0.01
	N Vitamin ≥30	0.40±0.01	*P*<0.001	*P*=0.002

P=Significance of differences with the control group according to the corresponding Vitamin D level; P_1=Significance of differences with the Vitamin D level <30 ng/mL in the corresponding group. AO=Aorta; LA=Left atrium; EF=Ejection fraction; PWT=Posterior wall thickness; IVSd=Intra-ventricular septum diastole; LVM=Left ventricular mass; LVMI=LVM index; RWT=Relative wall thickness, Echo-CG=Echocardiographic

in general) and over such with normal BMI, that accompanied by Vitamin D deficiency, so we hypothesized that might led to LVH and LVMI increase.

A linkage between LVH, LVMI, and parathormone has been demonstrated in some clinical and experimental studies. PTH was found to be associated with LVMI in patients with primary hyperparathyroidism, end-stage renal disease with secondary hyperparathyroidism, EAH, after aortic valve replacement. The relation between PTH and LVH was not only shown in disease conditions but also in the general population and elderly people.[29] We found and confirmed the direct relationship (correlation) between serum PTH and LVMI (r = 0.34; P = 0.045) in hypertensive women with T-allele of GNB3 (rs5443) (according to correlation analysis data), but no statistically reliable dependence was established of the average serum PTH values and separate Echo-CG parameters (according to Student's *t*-test or the Wilcoxon-Mann–Whitney *U*-test). The reason is different statistical analysis.

The genetic polymorphisms *AGT* (rs4762) and *GNB3* (rs5443) as unmodifiable markers could be used for LVH prediction in EAH patients in conjunction with ionized calcium and 25-hydroxyvitamin D, which might help to improve early diagnostic strategy in detecting HMOD in hypertensive patients. The Vitamin D deficiency in our study is accompanied with LVH and LVHI increase. Therefore, Vitamin D supplement might be recommended to the treatment in clinical practice in case of Vitamin D deficiency accompanied by LVH.

CONCLUSION

T-allele of the AGT (rs4762) and GNB3 (rs5443) genes are associated with myocardial structure changes in hypertensive patients: thicker PWT, IVSd, and higher LVMI in women for GNB3 (rs5443) gene.

Hypovitaminosis D in EAH patients is accompanied by LV remodeling: larger LA size, LVM and LVMI in women, with lower EF.

Hypocalcemia in our study links to the smaller wall thickness of hypertrophied LV myocardium in EAH individuals than in the case of its normal concentration, as well as a lower LVMI and better LV contractile function, which confirms the hypothesis of active involvement of calcium in the process of myocardial remodeling and hypertrophy.

Table 6: Echocardiography parameters depending on the ionized calcium Ca^{2+} level, M±m

Echo-CG values	Ca^{2+} concentration in the control group (mmol/L)		Ca^{2+} concentration in the study group (mmol/L)	
	Ca^{2+} levels	Results	↓ Ca^{2+} <1.12	N Ca^{2+} ≥1.12
Ao (cm)	↓ Ca^{2+} <1.12	2.34±0.08	3.37±0.14	3.55±0.12
	N Ca^{2+} ≥1.12	2.69±0.11	*P*<0.001	*P*<0.001
		P_1=0.034		
LA (cm)	↓ Ca^{2+} <1.12	2.50±0.11	4.13±0.12	4.14±0.11
	N Ca^{2+} ≥1.12	2.61±0.13	*P*<0.001	*P*<0.001
EF (%)	↓ Ca^{2+} <1.12	70.0±0.56	63.50±1.04	60.35±1.15
	N Ca^{2+} ≥1.12	69.0±0.82	*P*=0.002	*P*<0.001
				P_1=0.046
PWT (cm)	↓ Ca^{2+} <1.12	0.78±0.02	1.13±0.02	1.18±0.02
	N Ca^{2+} ≥1.12	0.77±0.03	*P*<0.001	*P*<0.001
				P_1=0.048
IVSd (cm)	↓ Ca^{2+} <1.12	0.76±0.02	1.16±0.015	1.21±0.02
	N Ca^{2+} ≥1.12	0.80±0.02	*P*<0.001	*P*<0.001
				P_1=0.049
LVM (g)	↓ Ca^{2+} <1.12	91.91±5.32	301.14±18.02	298.30±12.77
	N Ca^{2+} ≥1.12	91.45±7.67	*P*<0.001	*P*<0.001
LVMI (g/m^2)	↓ Ca^{2+} <1.12	48.27±2.14	139.73±3.22	150.01±5.12
	N Ca^{2+} ≥1.12	47.73±3.84	*P*<0.001	*P*<0.001
				P_1=0.052
RWT	↓ Ca^{2+} <1.12	0.40±0.01	0.42±0.01	0.45±0.01
	N Ca^{2+} ≥1.12	0.39±0.01	*P*<0.001	*P*<0.001
				P_1=0.017

P=Significance of differences with the control group according to the corresponding Ca^{2+} level; P_1=Significance of differences with the Ca^{2+} level <1.12 mmol/L in the corresponding group. AO=Aorta; LA=Left atrium; EF=Ejection fraction; PWT=Posterior wall thickness; IVSd=Intra-ventricular septum diastole; LVM=Left ventricular mass; LVMI=LVM index; RWT=Relative wall thickness; Echo-CG=Echocardiographic

Data availability statement

The data that support the findings of this study are available from the corresponding author, Voroniuk Kseniia, upon reasonable request.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest

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