ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

LncRNA-GAS5 and β-Catenin as Independent Predictors of Asymptomatic Organ Damage in Nondiabetic Hypertensive Patients

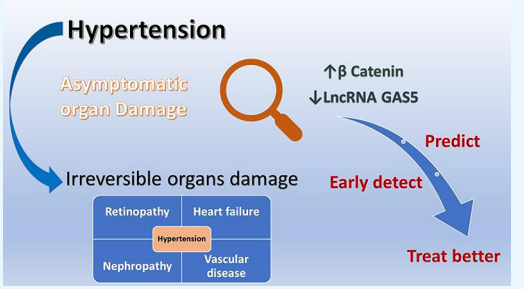
Marwa M. Esawy, Amir Abd-elhameed, Ahmed F. Gomaa, Shereen A. Baioumy, Moataz A. ElKot, Mohammed A. Hegab, Ahmed S. Alazzouni,* Felwa A. Thagfan, Rewaida Abdel-Gaber, Mohamed A. Dkhil, and Marwa A. Shabana

Cite This: *ACS Omega* 2023, 8, 6009−6015

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Hypertension is a serious medical condition that can increase the risk of developing heart, brain, kidney, and other diseases. Many asymptomatic hypertension patients experience asymptomatic organ damage (AOD). The purpose of this study was to determine the roles of LncRNA-GAS5 and β-catenin in predicting AOD in hypertensive nondiabetic patients. This study included 256 subjects, 128 hypertension patients (75 of whom had AOD, and 53 of whom did not) and 128 healthy controls. qRT-PCR was used to assess LncRNA-GAS5, and ELISA was used to assess β-catenin. The LncRNA-GAS5 expression level was decreased in hypertensive patients compared to controls (p-value < 0.001). On the other hand, β-catenin levels showed higher levels in the patients in comparison with controls (p-value < 0.001). A 0.38-fold change in LncRNA-GAS5 expression predicted AOD with 86.6% sensitivity and 88.7% specificity. β-Catenin > 80.5 pg/mL predicted AOD with a sensitivity of 82.6% and specificity of 69.8%. LncRNA-GAS5 expression was a better diagnostic predictor of AOD than β-catenin. According to multivariate logistic regression analysis, decreased LncRNA-GAS5 expression independently increased the risk of AOD (adjusted odds ratio = 0.03 (95% CI: 0.01−0.1) (p < 0.001). Furthermore, elevated β-catenin levels may be an independent risk factor for AOD (adjusted odds ratio = 14.3 (95% confidence interval, 3.3−61.9) (p < 0.001). Collectively, in hypertensive patients, LncRNA GAS5 and β-catenin can distinguish patients with AOD from those who do not have AOD. LncRNA GAS5 and β-catenin can be used as independent predictors of AOD in hypertensive patients.



1. INTRODUCTION

Hypertension is a serious medical condition that can increase the risk of developing heart, brain, kidney, and other diseases. As a result, hypertension is a major contributor to premature death globally.[1] It is a multifactorial disease influenced by genetic, environmental disorders. According to the most current World Health Organization (WHO) data, less than 20% of the estimated 1.13 billion people who have hypertension have the condition under control.[2] Due to its complex nature and unidentified cause, hypertension continues to be one of the most difficult disorders to study and treat despite years of effort. This intensifies the need for additional research to determine the molecular signaling that hypertension is caused by.[3] Endothelial dysfunction, atherosclerotic changes, and target organ damage are frequent symptoms of hypertension. Many asymptomatic and/or untreated hypertension patients experience asymptomatic organ damage (AOD).[4]

Despite the widespread assumption that most of the genetic information is transferred to proteins via mRNAs that encode proteins, current research indicates that most mammalian genomes are transcribed into noncoding (nc)RNA, which does not encode a protein.[5,6] Long noncoding (lnc) RNAs have the capacity to regulate gene expression at several different levels and in a more complex manner than miRNAs.[7] LncRNAs, including their potential as biomarkers and therapeutic targets, have just lately caught the attention of researchers, and still little is known about them.[8] Many diseases are caused by disruptions in the gene expression of lncRNAs. According to the lncRNA disease database, over 900 lncRNAs are involved in various disorders, with over 205,959 correlations with cardiovascular diseases (CVDs).[9] LncRNAs have been identified as possible therapeutic targets for CVDs because of their aberrant expression in heart disorders.[6]

Among other ncRNAs, the growth arrest-specific 5 (GAS5) gene can produce a lncRNA.[10] Screening for highly expressed genes in growth-arrest cells led to the discovery of GAS5, a tumor suppressor gene that is mapped to chromosome 1q25.[11]

Received: December 14, 2022
Accepted: January 19, 2023
Published: February 1, 2023

Cardiovascular problems have been associated with lncRNA GAS5 in the literature.[6] lncRNA GAS5 plays a role in controlling arterial remodeling and blood pressure. In vitro and in vivo, GAS5 controls the function of vascular smooth muscle cells and endothelial cells GAS5 interacts with β-catenin through a mechanism that influences nuclear translocation of β-catenin, which modulates β-catenin signaling activity.[12] Therefore, GAS5 intervention is a promising therapeutic approach for the management of vascular remodeling.[13]

Multiple lines of research have suggested that the Wingless-related integration (Wnt)/β-catenin pathway plays a crucial role in inducing cardiac hypertrophy, myocardial fibrosis, and hypertension.[14] Several potential Wnt/β-catenin signaling pathway regulators have been identified over time, and the Wnt pathway is now being considered as a therapeutic target.[15] Even though Wnt/β-catenin signaling is a desirable target in clinical trials, a more comprehensive assessment of various patients can be used to optimize and direct treatment.[16] The Wnt/β-catenin pathway is heavily regulated by lncRNAs, with the majority of lncRNAs either directly or indirectly increasing β-catenin stability and activation.[17] There are no studies in the literature on the relationship between lncRNA GAS5 and β-catenin and AOD in hypertensive patients. As a result, the purpose of this study was to evaluate the roles of LncRNA-GAS5 and β-catenin in predicting AOD in hypertensive nondiabetic patients, as well as their clinical associations.

2. SUBJECTS AND METHODS

2.1. Study Design. Between June 2022 and October 2022, the study was carried out at Zagazig University Hospitals. The Institutional Review Board (IRB) of the Faculty of Human Medicine at Zagazig University (IRB#10007) approved the study's protocol before it could be carried out. For their participation in the study, every subject signed a written informed consent form.

2.2. Subjects. The sample size was calculated using the Epi Info program 6 (Atlanta, Georgia, USA), which used a pooled standard deviation of 0.57 from a previous work by Wang et al.[12] and an assumption of a 0.2-fold variation in gene expression. This study included 256 subjects, 128 hypertension patients and 128 healthy controls. Subjects were enrolled consecutively. The AOD diagnosis was based on the presence of grade III retinopathy, left ventricular hypertrophy, abnormal carotid intima-media thickness (CIMT), and microalbuminuria. This study included 128 adult patients who had previously been diagnosed with hypertension, 75 of whom had AOD, and 53 of whom did not. Patients with secondary hypertension, dyslipidemia, diabetes, kidney illness, cardiovascular disease, cancer, liver diseases, or rheumatic diseases were not included (Figure 1). The patients were evaluated by taking comprehensive history. Clinical examinations included body mass index and blood pressure measurements were performed.

2.3. Methods. *2.3.1. Fundoscopic Examination.* All patients with hypertension were examined after pupil dilatation to look for signs and staging of hypertensive retinopathy using an indirect ophthalmoscope or + 90 noncontact lenses. Retinopathy staging was performed using the Keith−Wagener−Baker (KWB) system.[18]

2.3.2. Echocardiographic Examination. The GE Vivid E9 (model GA 091568, Norway) was used for echocardiography, with a 5 MHz transducer that included color flow, pulsed wave, continuous wave Doppler, and pulsed-wave TDI. By using the Teicholz method, the left ventricular ejection fraction (LVEF) was evaluated. The parasternal long-axis view should be used to take linear internal measurements of the left ventricle and its walls. Values should be accurately measured at or just below the level of the mitral valve leaflet tips, perpendicular to the left ventricle long axis.[19]

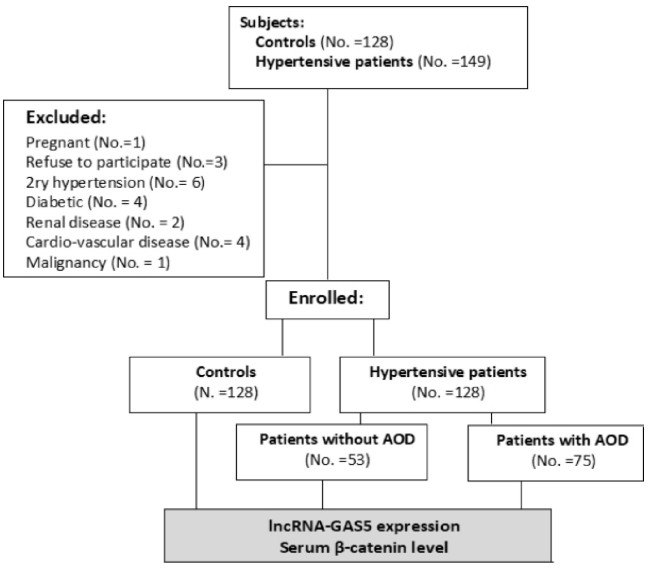


Figure 1. Study flowchart.

Using the recommended formula for the calculation of the left ventricle mass (LVM) from the left ventricle linear dimensions based on modeling the left ventricle as a prolate ellipse of rotation, the LVM was determined as follows: LVM (g) = 0.8(1.04(LVIDD + IVST + PWT)3 LVIDD3) + 0.6.[20] The left ventricle mass index (LVMI) was calculated by the following formula: LVMI = LVM/body surface area. For a case to be classified as having left ventricular hypertrophy, LVMI must be greater than 115 g/m^2 for men and 95 g/m^2 for women.[21]

2.3.3. Carotid Ultrasonography. B-mode ultrasonography was used to measure CIMT using a 10 MHz linear transducer (Philips HD7, Rothell, WA, USA). The probe was placed in the anterolateral position, while the individuals were lying supine for the examination. All IMT measurements were performed along a 1 cm portion of the common carotid artery, close to the carotid bulb, at the location of maximal thickness on the far wall of the artery. After the image was frozen, electronic calipers were used to take the measurements. Three readings of the CIMT were taken, and the mean value was used for statistical analysis. CIMT values of more than 0.9 mm are considered abnormal.[22]

2.3.4. Microalbuminuria. Patients were asked to collect 24 h urine samples; 24 h urine microalbumin was measured via the immunoturbidimetric method using the Tina-quant Albumin Gen. 2 kit on a cobas 6000 modular analyzer (Roche Diagnostics, Mannheim, Germany).

2.3.5. Serum β-Catenin Level. Whole blood was drawn and placed in a plastic Vacutainer plain tube (Becton, Dickinson and Company, Franklin Lakes, NJ). The tube was centrifuged for 10 min at 1200 × *g* after 30 min of blood collection. The 1.5 mL sterile microcentrifuge tubes with the aliquoted serum were stored at −80 ° C until the β-catenin measurement. The serum β-catenin level was measured by a human catenin beta-1 enzyme-linked immunosorbent assay kit (ELISA) [cat. no.: CSB-E08963h] (CUSABIO, Wuhan, China) following the

https://doi.org/10.1021/acsomega.2c07960
ACS Omega 2023, 8, 6009−6015

Table 1. Characteristics of Hypertensive Patients[a]

parameters	hypertensive patients (no.: 128)		p
	patients with AOD (no.: 75)	patients without AOD (no.: 53)	
age, years	54 [32−67]	50 [41.64]	0.024*
gender: male/female	42/33 (56/44)	32/21 (60.4/39.6)	0.62
smoking	20 (26.7)	13 (24.5)	0.79
BMI, kg/m^2	28.6 [22.2−33.3]	28.8 [24.1−33.5]	0.49
duration of hypertension, years	4 [1−8]	3 [1−7]	0.047*
antihypertensive drugs	63 (84)	43 (81.1)	0.67
SBP, mmHg	130 [110−155]	120 [110−135]	<0.001*
DBP, mmHg	80 [65−105]	75 [65−95]	0.012*
microalbuminuria (mg/d)	26 [4−88]	5.6 [3−14]	<0.001*
retinopathy			
none	44 (58.7)	43 (81.1)	0.023*
grade I	16 (21.3)	8 (15.1)	
grade II	10 (13.3)	2 (3.8)	
grade III	5 (6.7)	0	
LVEF, %	60 [54−68]	63 [57−72]	<0.001*
LVMI, g/m^2	77 [65−120]	65 [50−88]	<0.001*
CIMT, mm	0.8 [0.5−1]	0.6 [0.4−0.75]	<0.001*

[a]BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVEF: left ventricular ejection fraction; LVMI: left ventricle mass index; CIMT: carotid intima-media thickness test. Data are expressed as median [range] or number (%). *, significant.

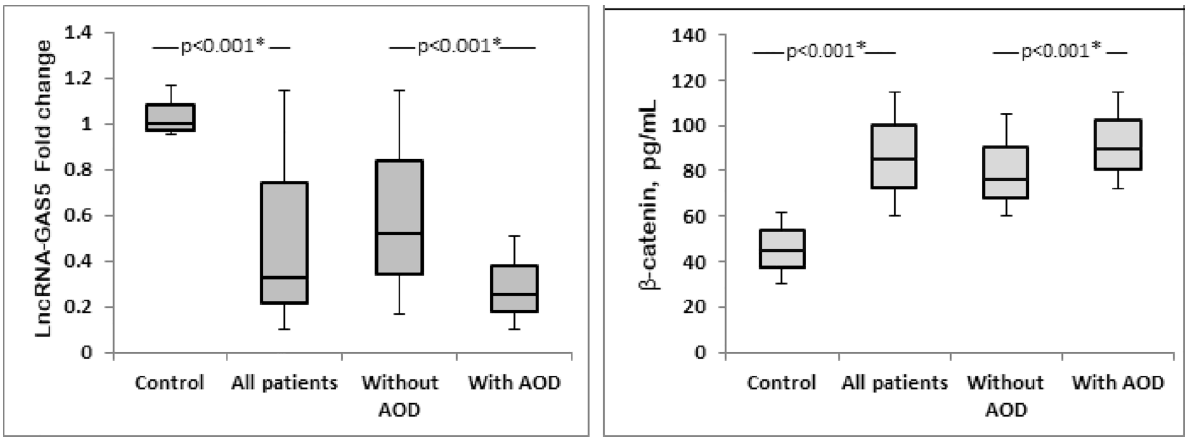


Figure 2. Real-time RT-PCR analysis of lncRNA-GAS5 and β-catenin. Signals for genes of interest were normalized to GAPDH signals, and relative expression is given as a fold increase compared to control.

recommendations provided by the manufacturer. The plates were read using the Sunrise absorbance reader (Männedorf, Switzerland). Values for serum β-catenin were reported in pg/mL. The intra- and inter-assay coefficients of variations for this kit were 8 and 12%, respectively.

2.3.6. LncRNA-GAS5 Expression. Using the miRNeasy Serum/Plasma Kit, total RNA was isolated from plasma in line with the manufacturer's instructions (QIAGEN, GmbH, Hilden, Germany). RNA purity and concentration were assessed using a NanoDrop-2000 spectrophotometer (Thermo Scientific, USA). Then, using the miScript RT II kit from QIAGEN GmbH, Hilden, Germany, reverse transcription of total RNA to complementary DNA (cDNA) was carried out. Each reaction was carried out using 1 μg of extracted RNA and miScript HiFlex Buffer. The reverse transcription was performed using the Gene Amp PCR System 9700 thermocycler (Perkin Elmer, Singapore). The thermal cycler has the following settings: 37 °C for 60 min and 95 °C for 5 min. Until analysis, the cDNA was stored at a temperature of −80 °C.

Using the Stratagene Mx3005P qPCR System (Agilent Technologies, Germany) and the miScript SYBR Green PCR Kit from QIAGEN, GmbH, Hilden, Germany, as directed by the manufacturer, the expression level of LncRNA-GAS5 was measured. The thermal profile consisted of a 15 min initial incubation at 95 ° C, followed by 40 cycles of 15 s at 94 ° C and 1 min at 60 ° C. Cycle threshold was used to express fluorescence measurement. The melting curve was checked for nonspecific florescence signals. The expression of LncRNA-GAS5 was normalized by the expression level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The fold change equation of $2^{-\Delta\Delta CT}$ was estimated to calculate the relative expression levels. The used primers (Metabion international AG, Planegg/Steinkirchen, Germany) for LncRNA-GAS5 detection were forward, 5′-AGCTGGAAGTTGAAATGG-3′; reverse, 5′-CAAGCCGACTCTCCATACC-3′. GAPDH-specific primers were forward, 5′-GAAGGTGAAGGTCG-GAGTC-3′; reverse, 5′-GAAGATGGTGATGGGATTTC-3′.

2.4. Statistical Analysis. The data were checked using the Shapiro−Wilk test; a nonparametric distribution was found.

https://doi.org/10.1021/acsomega.2c07960
ACS Omega 2023, 8, 6009−6015

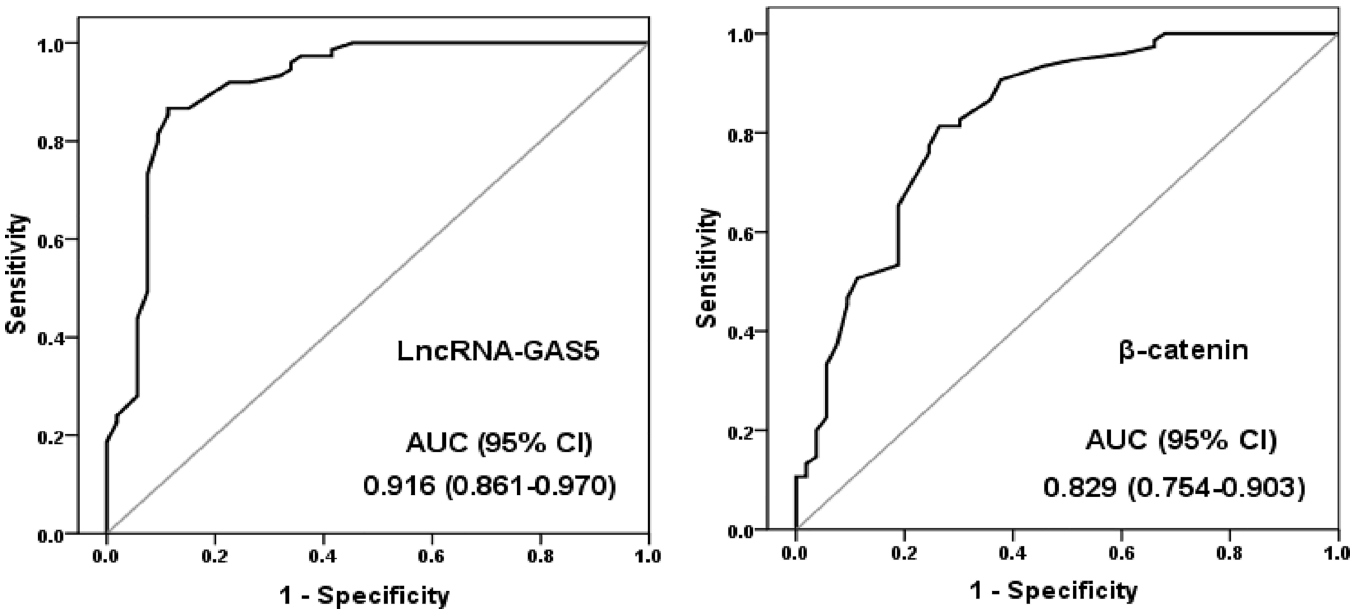


Figure 3. ROC curve analysis of the prediction of AOD.

The Mann−Whitney and chi-squared tests were used to compare parameters. The strength of the association was evaluated using the Spearman's correlation test. The effectiveness of the laboratory tests was evaluated using a receiver operator characteristic (ROC) curve. The 95% confidence interval (CI) and the area under the ROC curve (AUC) were calculated. The best cutoff point was determined using the highest Youden's index. To describe the risk factors, the odds ratio (OR) was computed using logistic regression analysis. Statistical significance was defined as a p-value less than 0.05. The statistical program utilized was SPSS 20 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

There were 256 participants in the study, with 128 hypertension patients (74 males and 54 females; median age: 51 [32−67] years) and 128 controls (71 males and 57 females; median age: 50 [30−66] years). The demographic data between the two groups did not significantly differ. Seventy-five hypertensive patients were reclassified as not having AOD, while 53 patients were categorized as having AOD. Table 1 lists the demographic, clinical, and laboratory findings for patient groups.

The LncRNA-GAS5 expression level was decreased in hypertensive patients compared to controls (p-value < 0.001). On the other hand, β-catenin levels showed higher levels in the patients in comparison with controls (p-value < 0.001) (Figure 2). The role of markers in detecting hypertension was assessed by ROC curve analysis. The lncRNA-GAS5 and β-catenin showed ROC-AUC values of 0.984 (95% CI: 0.964−1.004) and 0.999 (95% CI, 0.998−1.001), respectively. The lncRNA-GAS5 cut-off value was 0.92, with a sensitivity of 96.9% and a specificity of 100%. At the cut-off level of 62.5 pg/mL, the sensitivity of β-catenin is 98.4% with a specificity of 100%. So, β-catenin had higher performance characteristics in differentiate healthy individuals from hypertensive patients.

The median of lncRNA-GAS5 expression was significantly lower in patients with AOD in comparison to patients without AOD (0.25-fold change vs 0.52-fold change; $p < 0.001$). The median of β-catenin was significantly higher in the AOD group in comparison to patients without AOD (90 pg/mL vs 76 pg/mL; $p < 0.001$) (Figure 2).

The LncRNA-GAS5 expression level of <0.38-fold change (Youden index = 0.75) predicted AOD with a sensitivity of 86.6% and specificity of 88.7%. A β-catenin of >80.5 pg/mL (Youden index = 0.52) predicted AOD with a sensitivity of 82.6% and a specificity of 69.8%. LncRNA-GAS5 expression was a better diagnostic predictor of AOD than β-catenin (Figure 3).

The LncRNA-GAS5 expression level was negatively correlated with age, duration of hypertension, SBP, DBP, retinopathy, microalbuminuria level, LVEF, LVMI, CMIT, and β-catenin ($P < 0.05$). However, the β-catenin level was positively correlated with DBP, microalbuminuria level, LVEF, LVMI, and CMIT ($P < 0.05$) (Table 2).

The age of patients with AOD was higher than that of those without AOD. Also, the duration of hypertension was

Table 2. Correlation Analysis of the Studied Markers[a]

parameters	LncRNA-GAS5		β-catenin	
	r_s	p	r_s	p
age	−0.27	0.002*	0.1	0.46
BMI	−0.01	0.94	0.02	0.83
duration of hypertension	−0.19	0.03*	0.12	0.19
SBP	−0.39	<0.001*	0.16	0.08
DBP	−0.3	<0.001*	0.18	0.03*
microalbuminuria	−0.46	<0.001*	0.9	<0.001*
retinopathy	−0.34	<0.001*	0.12	0.19
LVEF	−0.31	<0.001*	0.22	0.012*
LVMI	−0.52	<0.001*	0.42	<0.001*
CIMT	−0.41	<0.001*	0.36	<0.001*
β-catenin	−0.42	<0.001*	1	

[a]rs: Spearman's rank correlation coefficient; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVEF: left ventricular ejection fraction; LVMI: left ventricle mass index; CIMT: carotid intima-media thickness test.

https://doi.org/10.1021/acsomega.2c07960
ACS Omega 2023, 8, 6009−6015

Table 3. Logistic Regression Analysis of AOD Risk Factors[a]

variables	univariate		multivariate	
	OR (95% CI)	p	AOR (95% CI)	p
age	0.94 (0.89−0.99)	0.04*	0.93 (0.83−1.03)	0.17
duration of hypertension	0.81 (0.66−0.98)	0.03*	0.7 (0.47−1.05)	0.09
SBP	0.92 (0.87−0.96)	<0.001*	0.94 (0.85−1.03)	0.19
DBP	0.93 (0.88−0.98)	<0.001*	1.03 (0.91−1.16)	0.69
LncRNA-GAS5	0.02(0.01−0.06)	<0.001*	0.03 (0.01−0.1)	<0.001*
β-catenin	12.1 (5.2−28.1)	<0.001*	14.3 (3.3−61.9)	<0.001*

[a]SBP: systolic blood pressure; DBP: diastolic blood pressure; OR: odds ratio; AOR: adjusted odds ratio; CI: confidence interval.

significantly higher in patients with AOD. So, age and duration of hypertension were included in the logistic regression analysis. Retinopathy, microalbuminuria, LVEF, LVMI, and CIMT findings were not included in the regression model, as they were used to identify the organs damage. The univariate logistic regression analysis showed that patient age, duration of hypertension, SBP, DBP, LncRNA-GAS5, and the β-catenin were associated with AOD. The significant risk factors were included in the multivariate model. Multivariate logistic regression analysis showed that the decreased LncRNA-GAS5 expression level independently increased the risk for AOD [AOR = 0.03 (0.01−0.1); $p < 0.001$]. Furthermore, increased β-catenin levels may be an independent risk factor for AOD [AOR = 14.3 (3.3−61.9); $p < 0.001$] (Table 3).

4. DISCUSSION

A systolic blood pressure of 140 mmHg or above, a diastolic blood pressure of 90 mmHg, or a history of hypertension were all considered to be indicators of hypertension.[23] Many patients with long-term high blood pressure may initially exhibit no symptoms but later develop hypertension-mediated organ damage (formerly known as target organ damage).[24] Left ventricular hypertrophy, microalbuminuria, retinopathy, and other conditions are examples of AOD.[25] The purpose of this study was to investigate the potential predictive value of lncRNA-GAS5 and β-catenin in hypertensive nondiabetic patients.

Our investigation identified several significant risk variables for AOD in hypertensive patients, including patient age, the duration of hypertension, SBP, and DBP. Similar findings have been made by Ates et al.,[26] who claim that factors such as sex, age, BMI, hypertension duration, mean 24 h SBP, and mean 24 h DBP can all independently predict an elevated risk of AOD. Additionally, Ateş et al.[27] discovered in a different study that the mean 24 h SBP, LVMI, microalbuminuria level, and retinopathy stage all correlated favorably. In all the patients being treated for hypertension, the 24 h SBP was found to be a predictor of such indications of AOD as LVMI, CIMT, and microalbuminuria.[27]

The involvement of the Wnt/β-catenin pathway in numerous biological processes, such as embryogenic development, adult tissue homeostasis, and wound healing, has received a lot of attention recently.[28] According to our findings, patients had higher levels of β-catenin than controls. Furthermore, elevated β-catenin levels may be a separate risk factor for AOD. Zhao et al.[14] discovered that canonic Wnt/β-catenin signaling was important in mediating cardiac hypertrophy in hypertension. While Kasacka et al.'s study[28] found a significant decrease in the expression of the gene encoding β-catenin in systemic hypertension and a significant increase in the expression of the tested gene in hypertensive rats compared to control groups, such a difference in findings could be explained by the study groups' different species.

It has become increasingly common to use circulating RNA in plasma or serum for noninvasive diagnostic purposes. LncRNAs are stable in human plasma, according to the information that is currently available. Circulating cell-free lncRNAs have been shown to act as biomarkers for cardiovascular and cancer disorders.[29] GAS5 has a high level of expression in adult tissues and is crucial for several biological functions.[30] The malfunctioning of vascular smooth muscle cells (VSMC) and endothelial cells (EC) is closely related to hypertension. It is possible that these cells' dysfunctional GAS5 expression will have an impact on how hypertension develops.[12] According to this study, hypertension patients' levels of lncRNA-GAS5 expression were lower than those of controls. According to Correia et al.,[6] vascular tone is controlled by lncRNAs, which are uniquely expressed in ECs and VSMCs cells and so play a role in the pathogenesis of arterial hypertension. The lncRNA GAS5 regulates vascular remodeling in hypertension, a process that determines the prognosis of the condition, by controlling the actions of ECs and VSMCs via β-catenin signaling.[6] The GAS5 knockdown mostly made hypertension worse in rat models that had already become hypertensive on their own due to an increase in blood arterial pressure, and it also made pathological arterial vascular remodeling, a typical complication in hypertensive people, worse.[12] LncRNA GAS5 showed differential expression in the arteries of hypertension patients as compared to healthy people, indicating a possible role for that lncRNA as a biomarker of hypertension.[6]

The current study found that individuals with AOD had considerably lower levels of lncRNA-GAS5 expression than patients without AOD. Also, β-catenin was significantly higher in the AOD group in comparison to patients without AOD. The LncRNA-GAS5 expression level was negatively correlated with retinopathy grads, and β-catenin was not correlated with retinopathy. These observations agreed with Jiang et al.[31] who reported that lncRNA GAS5 may suppress apoptosis and inflammation in retinal cells.

The LncRNA-GAS5 expression level was negatively correlated with LVEF and LVMI. However, the β-catenin level was positively correlated with LVEF and LVMI. These results corroborated Han et al.'s findings, which suggested that the lncRNA GAS5 may play a substantial role in the process of myocardial ischemia−reperfusion injury, exhibit significant expression changes, and play a role in myocardial hypertensive injury.[32] In both cardiomyocytes and interstitial fibroblasts, the main intracellular mediator of canonic Wnt signaling, β-catenin, was consistently up-regulated. Wnt/β-catenin activation may play an important role in mediating heart injury, as β-

https://doi.org/10.1021/acsomega.2c07960
ACS Omega 2023, 8, 6009−6015

catenin signaling suppression also significantly reduced blood pressure.[14] Myocardial hypertrophy may be caused by dysregulated Wnt/β-catenin pathway activity, specifically elevated levels of β-catenin, which can activate tissue fibrosis and promote cardiomyocyte proliferation.[33,34]

In the current study, the LncRNA-GAS5 expression level was negatively correlated with the microalbuminuria level. The β-catenin level was positively correlated with the microalbuminuria level. Overactivation of Wnt1/β-catenin signaling causes podocyte injury and epithelial−mesenchymal transition that results in renal injury and fibrosis.[35] This study revealed that the LncRNA-GAS5 expression level was negatively correlated with CMIT. However, the β-catenin level was positively correlated with CMIT. LncRNA GAS5 controlled the remodeling of several arteries, including the caudal, carotid, renal, and thoracic arteries.[12] During intimal thickening, Wnt/β-catenin signaling takes place in expanding vascular smooth muscle cells.[36]

The lncRNAs GAS5 and β-catenin can distinguish between patients with and without AOD. As a result, their measurement and monitoring may be helpful for hypertension patients' risk assessment and therapeutic decisions. To corroborate these findings, however, larger and more extensive research is needed. Our study had certain limitations, unfortunately. First, the study's patient population was handled by a single institution; hence, larger multicenter investigations are required to support the findings. Second, we only examined four indicators for subclinical target organ damage. Third, we were unable to assess the impact of antihypertensive medications. Finally, the dynamic changes in biomarkers with disease progression were not investigated.

5. CONCLUSIONS

In hypertensive patients, lncRNA GAS5 and β-catenin can differentiate patients with AOD from patients without AOD. Decreased lncRNA GAS5 and increased β-catenin level could be independent predictors of AOD in hypertensive patients.

AUTHOR INFORMATION

Corresponding Author

Ahmed S. Alazzouni − *Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo 11795, Egypt*; orcid.org/0000-0002-3392-802X; Email: drahmedalazzouni@gmail.com

Authors

Marwa M. Esawy − *Clinical Pathology Depart, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Amir Abd-elhameed − *Internal Medicine Department, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Ahmed F. Gomaa − *Internal Medicine Department, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Shereen A. Baioumy − *Microbiology and Immunology Department, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Moataz A. ElKot − *Cardiology Department, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Mohammed A. Hegab − *Ophthalmology Department, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Felwa A. Thagfan − *Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia*

Rewaida Abdel-Gaber − *Department of Zoology, Faculty of Science, King Saud University, Riyadh 11451, Saudi Arabia*

Mohamed A. Dkhil − *Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo 11795, Egypt; Applied Science Research Center, Applied Science Private University, Amman 11931, Jordan*

Marwa A. Shabana − *Clinical Pathology Depart, Faculty of Human Medicine, Zagazig University, Zagazig 44519, Egypt*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.2c07960

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R96), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia and also was supported by Researchers Supporting Project (RSP2023R25), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

(1) Madhur, M. S.; Elijovich, F.; Alexander, M. R.; Pitzer, A.; Ishimwe, J.; Van Beusecum, J. P.; Patrick, D. M.; Smart, C. D.; Kleyman, T. R.; Kingery, J.; et al. Hypertension: Do Inflammation and Immunity Hold the Key to Solving this Epidemic? *Circ. Res.* **2021**, *128*, 908−933.

(2) Melton, E.; Qiu, H. Interleukin-1β in Multifactorial Hypertension: Inflammation, Vascular Smooth Muscle Cell and Extracellular Matrix Remodeling, and Non-Coding RNA Regulation. *Int. J. Mol. Sci.* **2021**, *22*, 8639.

(3) Poulter, N. R.; Prabhakaran, D.; Caulfield, M. Hypertension. *Lancet* **2015**, *386*, 801−812.

(4) Yildirim, E.; Ermis, E.; Allahverdiyev, S.; Ucar, H.; Yavuzer, S.; Cengiz, M. Circulating miR-21 levels in hypertensive patients with asymptomatic organ damage. *Medicine* **2019**, *98*, e17297.

(5) Yu, B.; Wang, S. Angio-LncRs: LncRNAs that regulate angiogenesis and vascular disease. *Theranostics* **2018**, *8*, 3654−3675.

(6) Correia, C. C. M.; Rodrigues, L. F.; de Avila Pelozin, B. R.; Oliveira, E. M.; Fernandes, T. Long Non-Coding RNAs in Cardiovascular Diseases: Potential Function as Biomarkers and Therapeutic Targets of Exercise Training. *Non-Coding RNA* **2021**, *7*, 65.

(7) Statello, L.; Guo, C. J.; Chen, L. L.; Huarte, M. Gene Regulation by Long Non-Coding RNAs and Its Biological Functions. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 96−118.

(8) Jarroux, J.; Morillon, A.; Pinskaya, M. History, Discovery, and Classification of lncRNAs. *Adv. Exp. Med. Biol.* **2017**, *1008*, 1−46.

(9) Farsangi, S. J.; Rostamzadeh, F.; Sheikholeslami, M.; Jafari, E.; Karimzadeh, M. Modulation of the Expression of Long Non-Coding RNAs H19, GAS5, and MIAT by Endurance Exercise in the Hearts of Rats with Myocardial Infarction. *Cardiovasc. Toxicol.* **2021**, *21*, 162−168.

(10) Ji, J.; Dai, X.; Yeung, S. J.; He, X. The role of long non-coding RNA GAS5 in cancers. *Cancer Manag. Res.* **2019**, *Volume 11*, 2729−2737.

(11) Akiyama, T. Wnt/β-catenin signaling. *Cytokine Growth Factor Rev.* **2000**, *11*, 273−282.

(12) Wang, Y. N.; Shan, K.; Yao, M. D.; Yao, J.; Wang, J. J.; Li, X.; Liu, B.; Zhang, Y. Y.; Ji, Y.; Jiang, Q.; Yan, B. Long Noncoding RNA-GAS5: A Novel Regulator of Hypertension-Induced Vascular Remodeling. *Hypertension* **2016**, *68*, 736−748.

(13) Nusse, R.; Clevers, H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* **2017**, *169*, 985−999.
(14) Zhao, Y.; Wang, C.; Wang, C.; Hong, X.; Miao, J.; Liao, Y.; Zhou, L.; Liu, Y. An essential role for Wnt/β-catenin signaling in mediating hypertensive heart disease. *Sci. Rep.* **2018**, *8*, 8996.
(15) Liu, J.; Xiao, Q.; Xiao, J.; Niu, C.; Li, Y.; Zhang, X.; Zhou, Z.; Shu, G.; Yin, G. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. *Sig. Transduct. Target. Ther.* **2022**, *7*, 3.
(16) Kahn, M. Can we safely target the WNT pathway? *Nat. Rev. Drug Discovery* **2014**, *13*, 513−532.
(17) Guo, C. J.; Ma, X. K.; Xing, Y. H.; Zheng, C. C.; Xu, Y. F.; Shan, L.; Zhang, J.; Wang, S.; Wang, Y.; Carmichael, G. G.; et al. Distinct Processing of lncRNAs Contributes to Non-conserved Functions in Stem Cells. *Cell* **2020**, *181*, 621−636.e22.
(18) Fraser-Bell, S.; Symes, R.; Vaze, A. Hypertensive eye disease: a review. *Clin. Exp. Ophthalmol.* **2017**, *45*, 45−53.
(19) Kosaraju, A.; Goyal, A.; Grigorova, Y.; Makaryus, A. N. Left Ventricular Ejection Fraction. **2022**. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2022. PMID: 29083812.
(20) Barbieri, A.; Bursi, F.; Camaioni, G.; Maisano, A.; Imberti, J. F.; Albini, A.; De Mitri, G.; Mantovani, F.; Boriani, G. Echocardiographic Left Ventricular Mass Assessment: Correlation between 2D-Derived Linear Dimensions and 3-Dimensional Automated, Machine Learning-Based Methods in Unselected Patients. *J. Clin. Med.* **2021**, *10*, 1279.
(21) Lang, R. M.; Badano, L. P.; Mor-Avi, V.; Afilalo, J.; Armstrong, A.; Ernande, L.; Flachskampf, F. A.; Foster, E.; Goldstein, S. A.; Kuznetsova, T.; et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J. Am. Soc. Echocardiogr.* **2015**, *28*, 1−39.e14 e14.
(22) Pignoli, P.; Tremoli, E.; Poli, A.; Oreste, P.; Paoletti, R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* **1986**, *74*, 1399−1406.
(23) Teliewubai, J.; Bai, B.; Zhou, Y.; Lu, Y.; Yu, S.; Chi, C.; Li, J.; Blacher, J.; Xu, Y.; Zhang, Y. Association of asymptomatic target organ damage with secreted frizzled related protein 5 in the elderly: the Northern Shanghai Study. *Clin. Interv. Aging.* **2018**, *Volume 13*, 389−395.
(24) Williams, B.; Mancia, G.; Spiering, W.; Rosei, E. A.; Azizi, M.; Burnier, M. et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Kardiol Pol.* , *2019* (), 71−159.
(25) Wang, X.; Hao, G.; Chen, L.; Yang, Y.; Zhou, H.; Kang, Y.; Shaver, L.; Chen, Z.; Zheng, C.; Zhang, L.; et al. Hypertension-mediated organ damage and established cardiovascular disease in patients with hypertension: the China Hypertension Survey, 2012−2015. *J. Hum. Hypertens.* **2022**, *36*, 1092−1098.
(26) Ates, I.; Ozkayar, N.; Topcuoglu, C.; Dede, F. Relationship between oxidative stress parameters and asymptomatic organ damage in hypertensive patients without diabetes mellitus. *Scand. Cardiovasc. J.* **2015**, *49*, 249−256.
(27) Ateş, İ.; Altay, M.; Kaplan, M.; Ozkayar, N.; Toprak, G.; Alagüney, M. E.; Özkara, A. Relationship between socioeconomic level, and the prevalence of masked hypertension and asymptomatic organ damage. *Med. Sci. Monit.* **2015**, *21*, 1022−1030.
(28) Kasacka, I.; Piotrowska, Ż.; Domian, N.; Lewandowska, A. Wnt/β-catenin signaling in the adrenal glands of rats in various types of experimental hypertension. *Endocr. Connect.* **2022**, *11*, e220121.
(29) Wu, G. C.; Li, J.; Leng, R. X.; Li, X. P.; Li, X. M.; Wang, D. G.; Pan, H. F.; Ye, D. Q. Identification of long non-coding RNAs GAS5, linc0597 and lnc-DC in plasma as novel biomarkers for systemic lupus erythematosus. *Oncotarget* **2017**, *8*, 23650−23663.
(30) Williams, G. T.; Mourtada-Maarabouni, M.; Farzaneh, F. A critical role for non-coding RNA GAS5 in growth arrest and rapamycin inhibition in human T-lymphocytes. *Biochem. Soc. Trans.* **2011**, *39*, 482−486.
(31) Jiang, L.; Wang, C.; Shen, X. LncRNA GAS5 suppresses ER stress-induced apoptosis and inflammation by regulating SERCA2b in HG-treated retinal epithelial cell. *Mol. Med. Rep.* **2020**, *22*, 1072−1080.
(32) Han, Y.; Wu, N.; Xia, F.; Liu, S.; Jia, D. Long non-coding RNA GAS5 regulates myocardial ischemia-reperfusion injury through the PI3K/AKT apoptosis pathway by sponging miR-532-5p. *Int. J. Mol. Med.* **2020**, *45*, 858−872.
(33) Młynarczyk, M.; Kasacka, I. The role of the Wnt / β-catenin pathway and the functioning of the heart in arterial hypertension - A review. *Adv. Med. Sci.* **2022**, *67*, 87−94.
(34) Vallée, A.; Lévy, B. L.; Blacher, J. Interplay between the renin-angiotensin system, the canonical WNT/β-catenin pathway and PPARγ in hypertension. *Curr. Hypertens. Rep.* **2018**, *20*, 62.
(35) Guo, Q.; Zhong, W.; Duan, A.; Sun, G.; Cui, W.; Zhuang, X.; Liu, L. Protective or deleterious role of Wnt/beta-catenin signaling in diabetic nephropathy: An unresolved issue. *Pharmacol. Res.* **2019**, *144*, 151−157.
(36) Tsaousi, A.; Williams, H.; Lyon, C. A.; Taylor, V.; Swain, A.; Johnson, J. L.; George, S. J. Wnt4/β-catenin signaling induces VSMC proliferation and is associated with intimal thickening. *Circ. Res.* **2011**, *108*, 427−436.

https://doi.org/10.1021/acsomega.2c07960
ACS Omega 2023, 8, 6009−6015