

Original Article

Adducin 1 (alpha) Gly460Trp variant is associated with left ventricular geometry in Caucasians and African Americans: The HyperGEN Study

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Abstract: Normal left ventricular (LV) mass and geometry is required for optimal LV functioning. Abnormalities in either result in increased morbidity and mortality. The adducing 1 (alpha) gene (*ADD1*) Gly460Trp polymorphism has been associated with high blood pressure and increased plasma volume, both predictors of LV mass and function. In this cross-sectional study, we evaluate the association between this polymorphism and LV mass and geometry. LV mass, relative wall thickness (RWT), and systolic and diastolic parameters were measured using echocardiography in 3483 African American and Caucasian subjects from the Hypertension Genetic Epidemiology Network (HyperGEN). Analysis of covariance was used to estimate the polymorphism's association with echocardiograph parameters, stratified by race. The model was adjusted for age, diastolic and systolic blood pressure, glomerular filtration rate, smoking, low and high density lipoprotein cholesterol, urinary sodium, and body mass index. In Caucasians, the Trp allele was associated with higher ejection fraction (EF) ($P = .02$), fractional shortening (FS) ($P = .02$), and RWT ($P = .03$). In African Americans, the Trp allele was negatively associated with RWT ($P = .02$), but no association was found with EF ($P = .08$) or FS ($P = .09$). The polymorphism was not associated with diastolic function parameters in either racial group. We found no association of *ADD1* Gly460Trp with LV mass in Caucasians or African Americans; however, it was associated with unfavorable LV geometry (higher RWT) in Caucasians and favorable LV geometry (lower RWT) in African Americans after controlling for factors that would affect plasma volume.

Keywords: Adducin, genetic polymorphism, left ventricular mass, left ventricular geometry, hypertrophy

Introduction

Optimal functioning of the left ventricle requires normal left ventricular (LV) mass and geometry. Studies have shown that alteration in either LV mass [1, 2] or geometry [3-5] leads to impairment in LV function. Reduction of LV systolic function is associated with increased cardiovascular morbidity and mortality [6-10]. LV diastolic dysfunction is also a common correlate of hypertension and other cardiovascular morbidities. Therefore, preserving normal LV mass and geometry is an important clinical goal.

LV mass and geometry are, in part, under genetic influence, with heritability estimates for LV mass as high as 0.59 [11]. Candidate gene

studies have reported statistically significant associations between gene variants (e.g., in *TGFB1* [12], *NPPA* [13]) and LV hypertrophy as well as between variants (e.g., in *NPY2R*, *SFPR2*, *IL15* [14]) and other structural phenotypes. The adducin 1 (alpha) gene (*ADD1*) encodes a cytoskeletal protein involved in cell to cell contact [15], cell membrane transport [16], signal transduction [17], and promoting assembly of spectrin-actin complexes. A mutation in human *ADD1* results in the substitution of tryptophan (Trp) for glycine (Gly) at amino acid 460 (Gly460Trp, rs4961) [18]. Cusi *et al.* [18] have shown that the *ADD1* Gly460Trp polymorphism is associated with elevated blood pressure, plasma renin activity, and urinary tubular sodium reabsorption. Urinary sodium is a major

contributor to plasma volume [19, 20], and the downstream effect of increased plasma volume is higher blood pressure. The combination of volume expansion and elevated blood pressure can contribute to abnormalities of LV mass and geometry.

In this cross-sectional study, we evaluated the association between the *ADD1* Gly460Trp polymorphism and LV size and geometry. Previous studies have reported conflicting results regarding the association of this polymorphism with LV mass [21, 22], but their small sample sizes and differences in patient populations may explain the variable results. The Hypertension Genetic Epidemiology Network (HyperGEN) Study, with a sample size of 3483 individuals, is adequately powered to detect modest associations between the variant and echocardiographic LV parameters.

Materials and methods

Sample

The HyperGEN Study is a part of the Family Blood Pressure Program, designed to assess the genetic basis of hypertension in population-based samples. HyperGEN recruited three types of subjects: first, a sample of severe and mild hypertensive sibships with hypertension onset before age 60 without type 1 diabetes or renal failure; second, a random sample from the same base populations from which normotensive controls for genetic association and epidemiological analyses could be drawn; the third sample included the non-medicated adult offspring of the hypertensive siblings. The study populations were ascertained through population cohorts (Atherosclerosis Risk in Communities Study; the National Heart, Lung and Blood Institute Family Heart Study; and the Framingham Study). Hypertension was diagnosed according to treatment with one or more anti-hypertensive medications or average systolic blood pressure (SBP) ≥ 140 mm Hg or average diastolic blood pressure (DBP) ≥ 90 mm Hg from two clinic visits. The Genetics of Left Ventricular Hypertrophy Study is ancillary to HyperGEN and performed echocardiograms in 4 HyperGEN centers (Birmingham, AL; Forsyth County, NC; Minneapolis, MN; Salt Lake City, UT). Detailed information on study design and the recruitment strategy can be found in Williams *et al.* [23]. This study was approved by the centers' institu-

tional review boards, and all subjects gave informed consent.

Echocardiographic methods and derived variables

Echocardiograms were performed using a standardized protocol [24-26] with phased-array echocardiograph with M-mode, 2-dimensional, and color flow Doppler capability. The head of the examining table was elevated 30°, and a partial decubitus position was maintained. The parasternal acoustic window was used to record ≥ 10 consecutive beats of 2D and M-mode readings of LV internal diameter and wall thickness at or just below the mitral leaflet tips in long and short-axis views, long axis view of the mitral valve, color flow readings to search for mitral and aortic regurgitation, and M mode and 2D short- and long-axis views of the aortic root and left atrium. The apical window was used to record ≥ 10 cycles of 2- and 4-chamber images and color Doppler recordings to assess LV wall motion and valvular regurgitation.

Correct orientation of planes for imaging and Doppler recordings was verified according to standard procedures. LV internal dimensions and wall thickness were measured at end diastole and end systole according to American Society of Echocardiography recommendations [25]. When optimal orientation of LV M-mode readings could not be obtained, correctly oriented, linear, 2-dimensional measurements were made. Wall motion was assessed in parasternal long- and short-axis views and apical 2-, 3-, and 4-chamber views, with the left ventricle divided into 5 segments at the base, 5 at the papillary muscles, and 4 at the apex [27]. Doppler LV outflow tract measurements identified inflow and outflow profiles simultaneously for measurement of isovolumic reaction time (IVRT) from the aortic closure spike to the onset of mitral inflow. Echocardiographic measurements were made or confirmed by highly experienced physician investigators. All readers were blinded to subjects' clinical data.

LV mass was calculated using the anatomically validated formula [24]:

$$0.80 \times 1.04 [(IVSTd + LVIDd + PWTd) - (LVID)^3 - (LVIDd)^3] \times 0.6$$

LV mass index (LVMI) was calculated as LV mass/height²; LVMht^{2.7} was calculated as LV mass/height^{2.7} [28]. Relative wall thickness

(RWT) was calculated as twice the posterior wall thickness/LV internal radius at end-diastole. End-diastolic and -systolic LV volumes were calculated by the Teichholz method [29]. Myocardial contractile efficiency was assessed by examining the relation of midwall fractional shortening (MWS) to midwall circumferential end systolic stress (cESS) measured at the level of the LV minor axis [30-32]. MWS was calculated considering the epicardial migration of the midwall during systole; cESS was estimated at the midwall by the use of a cylindrical model [33]. MWS was expressed as a percent of the value predicted from cESS using an equation derived in normal subjects [30] and referred to as stress-corrected MWS. As 60% of subjects had diastolic filling measurements available at the leaflet tips and the rest had these measurements available at the annulus, the following equations were used to convert the diastolic filling parameters at the leaflet tips when the leaflet tip measurements were missing:

$$E_{\text{at tips}} = 0.84 \times E_{\text{at annulus}} + 23.3$$

$$A_{\text{at tips}} = 0.76 \times A_{\text{at annulus}} + 28.9$$

$$\text{deceleration time}_{\text{at tips}} = 0.56 \times \text{deceleration time}_{\text{at annulus}} + 106$$

Accepted protocols were used to calculate other echocardiographic measures of systolic and diastolic performance.

Other clinical measures

Average blood pressure was calculated using the second and third of three seated readings using an oscillometric blood pressure monitor (Dinamap 1846 SX/P; GE Healthcare, Waukegan, WI). Standardized assessments of body mass index (BMI), blood chemistries, and a lipid profile after a 12-hour fast were obtained. Diabetes was diagnosed by American Diabetes Association criteria (fasting blood glucose level >126 mg/dl or use of hypoglycemic medication). An overnight urine sample was obtained for urinary sodium; glomerular filtration rate (GFR) was estimated by the abbreviated Modification of Diet in Renal Disease equation [34, 35].

Genotyping

The *ADD1* Gly460Trp mutation was genotyped using a restriction fragment length polymor-

phism technique on polymerase chain reaction (PCR) products that were amplified from isolated genomic DNA. PCR products were exposed to the restriction endonuclease *Sau96 I*, and fragments were separated and identified using gel electrophoresis. Genotyping methods and quality control parameters for the method have been reported in more detail previously [36, 37].

Statistical methods

All statistical analyses were conducted using SAS (version 9.1). Data are presented as mean $\pm$ SD for continuous variables and as proportions for categorical variables. The χ^2 statistic was used to determine differences of categorical variables and to test for Hardy Weinberg equilibrium; Student's t-test was used to determine between-group differences of continuous variables. Because of the low minor allele frequency, the *ADD1* Gly460Trp genotypes were grouped as GlyGly and GlyTrp+TrpTrp for primary analyses. For secondary analyses, associations of echocardiographic variables (EF, FS, RWT) were also tested using the three genotypes. Comparison of means of echocardiographic measurements between genotype groups (i.e., GlyGly versus GlyTrp+TrpTrp) was made using one-way ANOVA in Caucasians and African Americans separately. Bivariate models of *ADD1* genotype group with potential covariates (age, race, gender, BMI, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), urinary sodium, GFR, DBP, SBP, presence of diabetes, hypertension, antihypertensive medication use, and smoking) were run, and those having more than 10% change in beta coefficient for *ADD1* genotype group were included in the final model. An ANCOVA model was used to adjust for potential confounding effects of these covariates. Potential interactions between the *ADD1* genotype group and covariates with echocardiographic variables were also tested. Log transformation was applied to renin, atrial natriuretic peptide, RWT, and MWS because of their skewed distribution. LVMI, cardiac output, cardiac output/body surface area values three times outside the interquartile range were deleted from all analyses. Tests of means across genotypes groups also were performed with mixed models, which adjust for the covariates and also take into account familial structure. Means of urinary sodium excretion and plasma

Table 1. Demographic and clinical characteristics of subjects by race, prevalence or mean $\pm$ SD

Characteristics	Caucasians (n=2004)	African Americans (n=1479)	P value
Age, yrs	55.8 $\pm$ 12.9	47.8 $\pm$ 12.7	<.0001
Women, %	52	64	<.0001
Hypertension, % present	65	73	<.0001
Diabetes, % present	14	21	<.0001
Antihypertensive medication, % using	58	62	.0373
Current smoker, %	45	53	<.0001
Body mass index, kg/m ²	29.3 $\pm$ 5.9	31.8 $\pm$ 7.5	<.0001
Systolic blood pressure, mm Hg	122.5 $\pm$ 19.9	128.7 $\pm$ 21.9	<.0001
Diastolic blood pressure, mm Hg	68.3 $\pm$ 10.4	73.8 $\pm$ 11.5	<.0001
Total cholesterol, mg/dl	198.3 $\pm$ 37.5	195.6 $\pm$ 40.0	.0454
Low density lipoprotein cholesterol, mg/dl	118.0 $\pm$ 32.1	120.3 $\pm$ 37	.0575
High density lipoprotein cholesterol, mg/dl	49.4 $\pm$ 14.8	53.7 $\pm$ 15.3	<.0001
Urinary sodium, mmol/L	98.5 $\pm$ 52.2	117.7 $\pm$ 60.6	<.0001
Adducin 1 (alpha), % with Trp allele	38	17	<.0001

Table 2. Trp allele frequency of adducin 1 (alpha) gene, stratified by center

	MA	MN	UT	NC		AL
	C (n=561)	C (n=518)	C (n=730)	C (n=195)	AA (n=497)	AA (n=982)
Adducin 1 (alpha), % with Trp allele	34	43	36	46	16	18

renin (data available for 919 subjects) were compared between GlyGly and GlyTrp+TrpTrp genotypes using ANCOVA adjusting for age, BMI, SBP, and diabetes, stratified by race. Because our dependant variables are strongly correlated, Bonferroni correction would have been overly conservative; therefore, we did not systematically apply multiple comparison criteria. Two-tailed *P* values of <.05 were considered significant.

Results

Among 3483 study participants, 58% were Caucasian, 57% were female, and 60% were taking antihypertensive medications. The distribution of *ADD1* Gly460Trp genotype in the study population was GlyGly, 71%; GlyTrp, 26%; and TrpTrp, 3%. **Table 1** shows the demographic and clinical characteristics of subjects, stratified by race. The frequency of the Trp allele was higher in Caucasians (38%) than African Americans (17%), (*P* < .0001). African Americans were

younger (mean age 48 $\pm$ 13 yr) than Caucasians (mean age 56 $\pm$ 13 yr; *P* < .0001). A greater proportion of African Americans had hypertension (73%) and diabetes (21%), with higher BMI (32 $\pm$ 8 vs. 29 $\pm$ 6 kg/m²), systolic blood pressure (129 $\pm$ 22 vs. 123 $\pm$ 20 mm Hg), diastolic blood pressure (74 $\pm$ 12 vs. 68 $\pm$ 10 mm Hg), and urinary sodium (118 $\pm$ 61 vs. 99 $\pm$ 52 mmol/L) than Caucasians. Caucasians had higher total cholesterol (198 $\pm$ 38 vs. 196 $\pm$ 40 mg/dL) but lower LDL-C and HDL-C than African Americans. The distribution of the *ADD1* Trp allele by field center is shown in **Table 2**. The distribution in each center and in the sample overall were in Hardy-Weinberg equilibrium.

Table 3 shows the results of the 1-way ANOVA comparing genotype groups with respect to echocardiographic measurements, stratified by race. In Caucasians the GlyTrp+TrpTrp genotype group had higher EF, FS, and RWT than the GlyGly genotype. In contrast, in African Americans the GlyTrp+TrpTrp genotype group had non-

Table 3. Age adjusted means $\pm$ SD of echocardiograph measurements, stratified by race (1-way ANOVA)

	Caucasians (n=2004)			African Americans (n=1479)		
	GlyGly	GlyTrp+TrpTrp	P*	GlyGly	GlyTrp+TrpTrp	P*
Structural variables						
LVMI, g/m ²	83.7 $\pm$ 0.6	83.2 $\pm$ 0.7	.6329	84.1 $\pm$ 0.7	82.9 $\pm$ 1.4	.4574
LVMht ^{2.7} , g/m ^{2.7}	40.2 $\pm$ 0.3	39.5 $\pm$ 0.4	.1649	40.8 $\pm$ 0.4	40.1 $\pm$ 0.8	.4324
RWT†	0.32 $\pm$ 0.004	0.33 $\pm$ 0.006	.0670	0.32 $\pm$ 0.005	0.31 $\pm$ 0.10	.0130
Systolic performance variables						
Cardiac index	2577.8 $\pm$ 17.2	2596.6 $\pm$ 21.9	.5017	2695.3 $\pm$ 17.3	2660.5 $\pm$ 37.8	.4045
EF	61.5 $\pm$ 0.2	62.4 $\pm$ 0.3	.0249	61.1 $\pm$ 0.2	60.3 $\pm$ 0.5	.1103
ESV	51.1 $\pm$ 0.6	48.5 $\pm$ 0.8	.0113	51.9 $\pm$ 0.7	54.3 $\pm$ 1.5	.1477
FS	33.5 $\pm$ 0.2	34.1 $\pm$ 0.2	.0261	33.2 $\pm$ 0.1	32.7 $\pm$ 0.3	.1161
MWS†	4.7 $\pm$ 0.004	4.7 $\pm$ 0.005	.7857	4.7 $\pm$ 0.004	4.7 $\pm$ 0.008	.5951
Diastolic performance variables						
IVRT, ms	82.3 $\pm$ 0.6	81.3 $\pm$ 0.7	.2277	78.4 $\pm$ 0.5	79.0 $\pm$ 1.0	.5541
MVA, cm/s	71.8 $\pm$ 0.6	72.1 $\pm$ 0.7	.7804	69.6 $\pm$ 0.6	69.4 $\pm$ 1.3	.8748
MVE, cm/s	75.9 $\pm$ 0.5	76.2 $\pm$ 0.7	.7546	78.5 $\pm$ 0.6	77.9 $\pm$ 1.2	.6773
MVE/MVA	1.13 $\pm$ 0.01	1.12 $\pm$ 0.02	.6019	1.21 $\pm$ 0.12	1.27 $\pm$ 0.04	.1417

LV, left ventricular; LVMI, LV mass/height²; LVMht^{2.7}, LV mass/height^{2.7}; RWT, relative wall thickness; EF, ejection fraction; ESV, end systolic volume; FS, fractional shortening; MWS, stress corrected midwall shortening; IVRT, iso-volumic reaction time; MVA, peak transmitral "A" velocity; MVE, peak transmitral "E" velocity.

*P values for F-test of means of means of corresponding echocardiographic measurements across the 2 genotypes.

†Natural log transformed data was used in ANOVA models.

statistically significant lower EF and FS but higher RWT than the GlyGly genotype. None of the other echocardiographic variables were associated with ADD1 genotype group in either African Americans or Caucasians in the 1-way ANOVA. Adjustment for additional potential confounding variables (DBP, SBP, GFR, smoking, LDL-C, HDL-C, urinary sodium, and BMI) for RWT, EF, and FS yielded virtually identical results (**Table 4**) for both the estimated means and the associated P value.

Discussion

This study shows that the presence of the Trp allele at position 460 of ADD1 is associated with higher FS, EF, and RWT in Caucasians. In contrast, African Americans with the Trp allele showed no associations with either EF or FS but

had an association with reduced RWT relative to the Gly homozygotes. There were no differences between genotype groups for unadjusted LV mass, LVMI, LVMht^{2.7}, MWS, or diastolic parameters (isovolumic reaction time, peak transmitral E or A wave velocities, or the transmitral E/A ratio) between the genotype groups in either Caucasians or African Americans.

The results of this study are in agreement with those of Castellano *et al.* [21] which showed no association of the ADD1 Gly460Trp polymorphism with LVMI (GlyGly, 96.5 $\pm$ 2.2; GlyTrp, 95.3 $\pm$ 4.9; TrpTrp, 108.5 $\pm$ 16.7), either before or after adjusting for confounding factors. Like our study, Castellano *et al.* also used a population-based sample with a genotype distribution similar to our study (GlyGly, 74.8%; GlyTrp, 20.7%; TrpTrp, 4.5%). Our results differ from

Table 4. Adjusted means (95% CI) in multivariate ANCOVA and mixed models, stratified by race

	Caucasians				African American			
	GlyGly	GlyTrp+TrpTrp	P value*	P value**	GlyGly	GlyTrp+Trp Trp	P value*	P value**
EF†	61.5 (61.05-61.9)	62.4 (61.8-63.0)	.0212	.0127	61.1 (60.7-61.6)	60.2 (59.2-61.1)	.0788	.1305
FS†	33.5 (33.2-33.8)	34.1 (33.7-34.5)	.0224	.0130	33.2 (32.9-33.5)	32.5 (31.9-33.2)	.0871	.1543
RWT‡	0.32 (0.32-0.33)	0.33 (0.32-0.33)	.0336	.0339	0.32 (0.31-0.32)	0.31 (0.30-0.31)	.0222	.0282

EF, ejection fraction; FS, fractional shortening; RWT, relative wall thickness; *P value for multivariate ANCOVA models.; **P value for mixed models; †Model adjusted for age, diastolic blood pressure, glomerular filtration rate, smoking, low density lipoprotein; ‡Model adjusted for age, urinary sodium, body mass index, systolic blood pressure, smoking, high density lipoprotein, glomerular filtration rate.

Winnicki *et al.*'s [22] which found a significant association ($P = .006$) of LVMI with the Gly460Trp polymorphism. However, their study employed multivariate analysis and was considerably smaller than ours ($n=162$ vs. 3483) [22]. The study population also differed: Winnicki *et al.* [22] studied mildly hypertensive Caucasian men, while our study population comprised men and women, Caucasians and African Americans, and hypertensive and normotensive individuals. Winnicki *et al.* [22] categorized genotype as GlyGly, GlyTrp, and TrpTrp and found that LVMI was only associated with the TrpTrp genotype, while we compared GlyGly vs. GlyTrp+TrpTrp genotype groups. Therefore, we performed a secondary analysis that used the three un-pooled genotypes. In Caucasians the associations with EF, FS, and RWT remained the same as in the pooled analysis [EF (GlyGly, 61.5 ± 0.2 ; GlyTrp, 62.3 ± 0.3 ; TrpTrp, 63.6 ± 0.9 ; $P = .02$); FS (GlyGly, 33.5 ± 0.2 ; GlyTrp, 34.0 ± 0.2 ; TrpTrp, 34.9 ± 0.6 ; $P = .3$); and RWT (GlyGly, 0.332 ± 0.004 ; GlyTrp, 0.332 ± 0.006 ; TrpTrp, 0.339 ± 0.01 ; $P = .08$)]. Conversely in African Americans EF and FS did not show significant associations in the comparable analysis, and RWT showed a marginal association ($P = .04$).

Changes in LV mass [38, 39] and geometry [39-41] are important predictors of cardiovascular events. LV hypertrophy (LVH) increases morbidity and mortality from myocardial infarction, stroke, and congestive cardiac failure. Blood pressure is an important contributor to LVH [42]. Epidemiologic studies have shown that both genetic and environmental factors [22, 43-46] play a significant role in LVH. Adducin is a cytoskeleton membrane protein, which pro-

motes binding of spectrin and actin; these actin-spectrin-based membrane proteins help to assemble integral membrane proteins and to couple them to cytoplasm proteins [47]. In the cell, this interaction of cytoskeletal and membrane proteins is essential for maintenance of cell polarity [48, 49] and regulation of ion transport [50, 51]. In vitro studies in rat kidney epithelial cells transfected with Milan hypertensive strains (MHS, missense mutations in α (F316Y) and β (Q529R) subunits of adducin [21]) have a significant increase in Na-K pump activity at V_{max} and Na-K pump units compared with cells carrying the Milan normotensive strains [16]. MHS also show an increase in enzymatic activity of outer medulla Na-K ATPase. Decreased urinary excretion of sodium causes an increase in intravascular volume which leads to an increase in volume overload [18] resulting in a change in LV geometry. Apart from this, the myocardium is rich in adducin; therefore, a polymorphism in this gene may have a direct effect on the myocardium [22].

Our study suggests an association in Caucasians between the Gly460Trp polymorphism and increased RWT but no association with measures of LV mass; this pattern (increased RWT, normal LV mass) is also referred to as concentric LV remodeling. Concentric remodeling is seen in groups with highest peripheral resistance; the normal LV mass in concentric remodeling reflects offsetting by volume underload of the effect of pressure overload [52]. In the HyperGEN Study, Province *et al.* have shown an association between higher blood pressure and presence of the Trp allele (OR=1.55, CI=1.03-2.34) in Caucasians [36]. No significant differences in urinary sodium excretion

have been associated with the Trp allele [53]; however, alterations in urinary sodium excretion have been associated with changes in plasma volume. Therefore, the mechanisms whereby the Trp allele raises blood pressure in a population, and how that leads to concentric remodeling as seen in our study population, needs further evaluation.

The presence of the Trp allele was not associated with any difference in MWS in either racial group. MWS is a more direct measure of myocardial contractile function than ejection fraction [30]. MWS might be subnormal or normal in the presence of normal or increased ejection fraction with higher RWT (as seen in our Caucasian population). MWS gives a superior estimate of myocardial function compared to EF especially in the presence of abnormal left geometry as indicated by increases in RWT in our study. Diastolic parameters (isovolumic relaxation time, mitral E and A velocities, and E/A ratio) did not differ across the genotype groups. These may be affected early in development of LV hypertrophy [38, 39, 54]; as LV mass did not differ across genotype groups, lack of Gly460Trp association with these parameters is not surprising.

The Trp allele was associated positively with RWT in Caucasians but negatively in African Americans. The lower RWT in African Americans with the Trp allele might reflect lack of association of the allele with blood pressure [55], since previous HyperGEN studies have shown the Trp allele to have a protective effect against hypertension traits in African Americans [36]. However, when we analyzed genotype specific differences in RWT in African Americans, we found that heterozygotes had the lowest RWT of all three genotypes ($P < .04$).

This study has important strengths, including population-based recruitment, standardized blood pressure and laboratory measurements, a large sample that included women and men and Caucasians and African Americans, as racial differences in *ADD1* associations have been described [36]. Second, we accounted for several clinical and demographic factors that have been shown to influence LV size and function [6, 31, 42]. This study also has limitations. Like all cross-sectional studies, ours provides evidence of association but cannot evaluate the temporality of the relationship. Because most

hypertensive participants in our study were taking antihypertensive medications, our findings may have been confounded by medication use. We did not take into account multiple testing, which may increase the probability of a false positive finding; however the Bonferroni correction for multiple testing assumes independence and, therefore, would have been an overcorrection since our dependent variables are strongly correlated. Although there was a consistency of the gene-phenotype association within the systolic function domain, suggesting that our findings are likely real, marginally significant associations should be interpreted with care.

In summary, our study found no association between the *ADD1* Gly460Trp variant and measures of LV mass. However, the Trp allele was associated with increased RWT and concentric remodeling in Caucasians and decreased RWT and favorable LV geometry in African Americans. Future genetic studies might find it opportune to examine *ADD1* Gly460Trp associations with LV structure and function in normotensive populations. Molecular studies may illuminate whether this polymorphism influences the myocardium directly or whether observed associations are a function of blood pressure and plasma renin activity.

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References

- [1] Wachtell K, Rokkedal J, Bella JN, Aalto T, Dahlof B, Smith G, Roman MJ, Ibsen H, Aurigemma GP and Devereux RB. Effect of electrocardiographic left ventricular hypertrophy on left ventricular systolic function in systemic hypertension (The LIFE Study). Losartan Intervention For Endpoint. Am J Cardiol 2001; 87: 54-60.
- [2] Guazzi MD, Fiorentini C and Barbier P. Assessment of the systolic function and contractility of the hypertensive left ventricle. J Clin Hypertens

- 1987; 3: 178-186.
- [3] Kimura E, Tabata T, Tanaka H, Harada K, Yamada H, Nomura M, Oki T and Ito S. Left ventricular geometry and myocardial contractility in patients with essential hypertension evaluated by myocardial velocity profile. *J Am Soc Echocardiogr* 2005; 18: 1222-1229.
- [4] de Simone G, Kitzman DW, Chinali M, Oberman A, Hopkins PN, Rao DC, Arnett DK and Devereux RB. Left ventricular concentric geometry is associated with impaired relaxation in hypertension: the HyperGEN study. *Eur Heart J* 2005; 26: 1039-1045.
- [5] Milani RV, Lavie CJ, Mehra MR, Ventura HO, Kurtz JD and Messerli FH. Left ventricular geometry and survival in patients with normal left ventricular ejection fraction. *Am J Cardiol* 2006; 97: 959-963.
- [6] Gosse P and Dallochio M. Left ventricular hypertrophy: epidemiological prognosis and associated critical factors. *Eur Heart J* 1993; 14 Suppl D: 16-21.
- [7] Benjamin EJ and Levy D. Why is left ventricular hypertrophy so predictive of morbidity and mortality? *Am J Med Sci* 1999; 317: 168-175.
- [8] Pierdomenico SD, Lapenna D, Bucci A, Manente BM, Cuccurullo F and Mezzetti A. Prognostic value of left ventricular concentric remodeling in uncomplicated mild hypertension. *Am J Hypertens* 2004; 17: 1035-1039.
- [9] Oda S, Yoshiyama M, Ichiba N, Kataoka T, Takekoto Y, Hozumi T, Takeuchi K and Yoshikawa J. The relationship between left ventricular geometric remodeling and cardiac function in essential hypertension. *Osaka City Med J* 2004; 50: 69-77.
- [10] Muesan ML, Salvetti M, Monteduro C, Bonzi B, Paini A, Viola S, Poisa P, Rizzoni D, Castellano M and Agabiti-Rosei E. Left ventricular concentric geometry during treatment adversely affects cardiovascular prognosis in hypertensive patients. *Hypertension* 2004; 43: 731-738.
- [11] Sharma P, Middelberg RP, Andrew T, Johnson MR, Christley H and Brown MJ. Heritability of left ventricular mass in a large cohort of twins. *J Hypertens* 2006; 24: 321-324.
- [12] Xu HY, Hou XW, Wang LF, Wang NF and Xu J. Association between transforming growth factor beta1 polymorphisms and left ventricle hypertrophy in essential hypertensive subjects. *Mol Cell Biochem* 335: 13-17.
- [13] Xue H, Wang S, Wang H, Sun K, Song X, Zhang W, Fu C, Han Y and Hui R. Atrial natriuretic peptide gene promoter polymorphism is associated with left ventricular hypertrophy in hypertension. *Clin Sci (Lond)* 2008; 114: 131-137.
- [14] Arnett DK, Devereux RB, Rao DC, Li N, Tang W, Kraemer R, Claas SA, Leon JM and Broeckel U. Novel genetic variants contributing to left ventricular hypertrophy: the HyperGEN study. *J Hypertens* 2009; In Press:
- [15] Kaiser HW, O'Keefe E and Bennett V. Adducin: Ca++-dependent association with sites of cell-cell contact. *J Cell Biol* 1989; 109: 557-569.
- [16] Tripodi G, Valtorta F, Torielli L, Chieriegatti E, Salardi S, Trusolino L, Menegon A, Ferrari P, Marchisio PC and Bianchi G. Hypertension-associated point mutations in the adducin alpha and beta subunits affect actin cytoskeleton and ion transport. *J Clin Invest* 1996; 97: 2815-2822.
- [17] Mische SM, Mooseker MS and Morrow JS. Erythrocyte adducin: a calmodulin-regulated actin-bundling protein that stimulates spectrin-actin binding. *J Cell Biol* 1987; 105: 2837-2845.
- [18] Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, Glorioso N, Lanzani C, Manunta P, Righetti M, Rivera R, Stella P, Troffa C, Zagato L and Bianchi G. Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. *Lancet* 1997; 349: 1353-1357.
- [19] Ledingham JM. Sodium retention and volume expansion as mechanisms. *Am J Hypertens* 1991; 4: 534S-540S.
- [20] Kurtz TW, Al-Bander HA and Morris RC, Jr. "Salt-sensitive" essential hypertension in men. Is the sodium ion alone important? *N Engl J Med* 1987; 317: 1043-1048.
- [21] Castellano M, Barlassina C, Muesan ML, Beschi M, Cinelli A, Rossi F, Rizzoni D, Cusi D and Agabiti-Rosei E. Alpha-adducin gene polymorphism and cardiovascular phenotypes in a general population. *J Hypertens* 1997; 15: 1707-1710.
- [22] Winnicki M, Somers VK, Accurso V, Hoffmann M, Pawlowski R, Frigo G, Visentin P and Palatini P. alpha-Adducin Gly460Trp polymorphism, left ventricular mass and plasma renin activity. *J Hypertens* 2002; 20: 1771-1777.
- [23] Williams RR, Rao DC, Ellison RC, Arnett DK, Heiss G, Oberman A, Eckfeldt JH, Leppert MF, Province MA, Mockrin SC and Hunt SC. NHLBI family blood pressure program: methodology and recruitment in the HyperGEN network. *Hypertension genetic epidemiology network. Ann Epidemiol* 2000; 10: 389-400.
- [24] Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I and Reichel N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol* 1986; 57: 450-458.
- [25] Sahn DJ, DeMaria A, Kisslo J and Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; 58: 1072-1083.
- [26] Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichel N, Sahn D, Schnittger I and et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989; 2: 358-367.

- [27] Shiina A, Tajik AJ, Smith HC, Lengyel M and Seward JB. Prognostic significance of regional wall motion abnormality in patients with prior myocardial infarction: a prospective correlative study of two-dimensional echocardiography and angiography. *Mayo Clin Proc* 1986; 61: 254-262.
- [28] de Simone G, Daniels SR, Devereux RB, Meyer RA, Roman MJ, de Divitiis O and Alderman MH. Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. *J Am Coll Cardiol* 1992; 20: 1251-1260.
- [29] Teichholz LE, Kreulen T, Herman MV and Gorlin R. Problems in echocardiographic volume determinations: echocardiographic-angiographic correlations in the presence of absence of asynergy. *Am J Cardiol* 1976; 37: 7-11.
- [30] de Simone G, Devereux RB, Roman MJ, Ganau A, Saba PS, Alderman MH and Laragh JH. Assessment of left ventricular function by the midwall fractional shortening/end-systolic stress relation in human hypertension. *J Am Coll Cardiol* 1994; 23: 1444-1451.
- [31] Shimizu G, Hirota Y, Kita Y, Kawamura K, Saito T and Gaasch WH. Left ventricular midwall mechanics in systemic arterial hypertension. Myocardial function is depressed in pressure-overload hypertrophy. *Circulation* 1991; 83: 1676-1684.
- [32] Devereux RB. Left ventricular geometry, pathophysiology and prognosis. *J Am Coll Cardiol* 1995; 25: 885-887.
- [33] Gaasch WH, Zile MR, Hoshino PK, Apstein CS and Blaustein AS. Stress-shortening relations and myocardial blood flow in compensated and failing canine hearts with pressure-overload hypertrophy. *Circulation* 1989; 79: 872-883.
- [34] Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F and Levey AS. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 2002; 39: 920-929.
- [35] Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N and Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; 130: 461-470.
- [36] Province MA, Arnett DK, Hunt SC, Leidencker-Foster C, Eckfeldt JH, Oberman A, Ellison RC, Heiss G, Mockrin SC and Williams RR. Association between the alpha-adducin gene and hypertension in the HyperGEN Study. *Am J Hypertens* 2000; 13: 710-718.
- [37] Bray MS, Li L, Turner ST, Kardina SL and Boerwinkle E. Association and linkage analysis of the alpha-adducin gene and blood pressure. *Am J Hypertens* 2000; 13: 699-703.
- [38] Levy D, Garrison RJ, Savage DD, Kannel WB and Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med* 1990; 322: 1561-1566.
- [39] Koren MJ, Devereux RB, Casale PN, Savage DD and Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med* 1991; 114: 345-352.
- [40] de Simone G, Devereux RB, Koren MJ, Mensah GA, Casale PN and Laragh JH. Midwall left ventricular mechanics. An independent predictor of cardiovascular risk in arterial hypertension. *Circulation* 1996; 93: 259-265.
- [41] Ghali JK, Liao Y and Cooper RS. Influence of left ventricular geometric patterns on prognosis in patients with or without coronary artery disease. *J Am Coll Cardiol* 1998; 31: 1635-1640.
- [42] Harnett JD, Kent GM, Barre PE, Taylor R and Parfrey PS. Risk factors for the development of left ventricular hypertrophy in a prospectively followed cohort of dialysis patients. *J Am Soc Nephrol* 1994; 4: 1486-1490.
- [43] Verhaaren HA, Schieken RM, Mosteller M, Hewitt JK, Eaves LJ and Nance WE. Bivariate genetic analysis of left ventricular mass and weight in pubertal twins (the Medical College of Virginia twin study). *Am J Cardiol* 1991; 68: 661-668.
- [44] Diet F, Graf C, Mahnke N, Wassmer G, Predel HG, Palma-Hohmann I, Rost R and Bohm M. ACE and angiotensinogen gene genotypes and left ventricular mass in athletes. *Eur J Clin Invest* 2001; 31: 836-842.
- [45] Devereux RB, Roman MJ, de Simone G, O'Grady MJ, Paranicas M, Yeh JL, Fabsitz RR and Howard BV. Relations of left ventricular mass to demographic and hemodynamic variables in American Indians: the Strong Heart Study. *Circulation* 1997; 96: 1416-1423.
- [46] Bella JN, MacCluer JW, Roman MJ, Almasy L, North KE, Best LG, Lee ET, Fabsitz RR, Howard BV and Devereux RB. Heritability of left ventricular dimensions and mass in American Indians: The Strong Heart Study. *J Hypertens* 2004; 22: 281-286.
- [47] Bennett V. Spectrin-based membrane skeleton: a multipotential adaptor between plasma membrane and cytoplasm. *Physiol Rev* 1990; 70: 1029-1065.
- [48] Hammerton RW, Krzeminski KA, Mays RW, Ryan TA, Wollner DA and Nelson WJ. Mechanism for regulating cell surface distribution of Na⁺,K⁺-ATPase in polarized epithelial cells. *Science* 1991; 254: 847-850.
- [49] Marrs JA, Napolitano EW, Murphy-Erdosh C, Mays RW, Reichardt LF and Nelson WJ. Distinguishing roles of the membrane-cytoskeleton and cadherin mediated cell-cell adhesion in generating different Na⁺,K⁺-ATPase distributions in polarized epithelia. *J Cell Biol* 1993; 123: 149-164.
- [50] Berdiev BK, Prat AG, Cantiello HF, Ausiello DA, Fuller CM, Jovov B, Benos DJ and Ismailov, II. Regulation of epithelial sodium channels by

- short actin filaments. *J Biol Chem* 1996; 271: 17704-17710.
- [51] Cantiello HF. Role of the actin cytoskeleton on epithelial Na⁺ channel regulation. *Kidney Int* 1995; 48: 970-984.
- [52] Ganau A, Devereux RB, Roman MJ, de Simone G, Pickering TG, Saba PS, Vargiu P, Simongini I and Laragh JH. Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. *J Am Coll Cardiol* 1992; 19: 1550-1558.
- [53] Kamitani A, Wong ZY, Fraser R, Davies DL, Connor JM, Foy CJ, Watt GC and Harrap SB. Human alpha-adducin gene, blood pressure, and sodium metabolism. *Hypertension* 1998; 32: 138-143.
- [54] Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Bartoccini C, Santucci A, Santucci C, Reboldi G and Porcellati C. Adverse prognostic significance of concentric remodeling of the left ventricle in hypertensive patients with normal left ventricular mass. *J Am Coll Cardiol* 1995; 25: 871-878.
- [55] Larson N, Hutchinson R and Boerwinkle E. Lack of association of 3 functional gene variants with hypertension in African Americans. *Hypertension* 2000; 35: 1297-1300.