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Altered Beta-2 Adrenergic Receptor Gene Expression in Human Clinical Hypertension

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Objectives: The beta-2 adrenergic receptor is involved in mediating vasodilatation via neurohumoral and sympathetic nervous system pathways. Alterations in beta-2 adrenergic receptor gene expression (mRNA transcription) may contribute to the hypertensive phenotype. Human gene expression in clinical phenotypes remains largely unexplored due to ethical constraints involved in obtaining human tissue. We devised a method to obtain normally discarded internal mammary artery tissue from coronary artery bypass graft patients. We then investigated differences in hypertensive and normotensive participants' beta-2 adrenergic receptor gene expression in this tissue. *Methods:* We collected arterial tissue samples from 46 coronary artery bypass patients in a surgical setting. Using 41 of the samples, we performed TaqMan real-time polymerase chain reaction (RT-PCR) and used the delta delta cycle threshold (ΔΔCt) relative quantitation method for determination of fold-differences in gene expression between normotensive and hypertensive participants. The beta-2 adrenergic receptor target was normalized to glyceraldehyde-phosphate dehydrogenase. *Results:* Participants with hypertension had significantly less-expressed beta-2 adrenoceptor gene (2.76-fold, $p < .05$) compared to normotensive participants. After Bonferroni correction, gene expression did not differ by race, gender, type/dose of β-blocker prescribed, positive family history of hypertension, or diagnosis of diabetes mellitus type 2. *Conclusions:* These data support the possibility of a molecular basis for impaired adrenoceptor-mediated vascular tone in hypertension. Modification and extension of this research is required.

Keywords: Beta-2 adrenergic receptor; gene expression; transcription; hypertension; coronary artery

Adrenergic receptors (AR) influence blood pressure (BP) via neurohumoral and sympathetic nervous system regulation. Alterations in ARs at cellular and molecular levels may lead to hypertension (HTN; Hindorf et al., 2005; Small, McGraw, & Liggett, 2003). The beta-2 adrenergic receptor (*ARB2*) mediates vasodilation in vascular smooth muscle as a target receptor for epinephrine and norepinephrine; when ARB2 protein function is impaired, HTN may ensue (Monopoli, Conti, Forlani, & Ognini, 1993).

Early protein-expression work showed that ARB2 density was positively correlated with mean arterial BP in lymphocytes of borderline and essential hypertensive

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participants (Brodde, Daul, O'Hara, Wang, & Bock, 1985) and in mononuclear cells across a wide range of participants with normal to increased BPs (Middeke, Remien, & Holzgreve, 1984). These protein expression studies added support to the hypothesis that impaired ARB2 relaxation was involved in the HTN disease process; however, a question remained as to whether or not genetics had an influence on the ARB2 density. Accordingly, Michel, Galal, Stoermer, Bock, and Brodde (1989) examined lymphocyte ARB2 density in children with positive and negative parental histories of HTN. They found no difference in receptor density between groups, implying nongenetic influences on ARB2 mechanisms in HTN. In more recent years, however, the genomic revolution has allowed more robust explorations into the possible molecular mechanisms in ARB2 density and HTN through gene association studies. Both positive and negative associations of *ARB2* gene variants (polymorphisms) with HTN have led to inconclusive evidence for alterations at the DNA level impacting the development of HTN (see reviews by Hantow et al., 2006, and Small et al., 2003).

The following approach can be applied to the study of AR involvement in disease: the genetic code (DNA) provides the template for transcription of messenger RNA (mRNA), which is then translated into protein (protein being the actual receptor, in this case). Transcriptional activity is generally thought to be influenced by environmental factors. In the case of the *ARB2* gene and HTN, for example, dietary sodium chloride intake is a mediator of *ARB2* gene expression in humans (Feldman, 2000). (An interesting side note: there is new evidence for possible genetic determinants of gene-expression traits that may change the way we view the interface between genetics and genomics in the future [Emilsson et al., 2008].) We hypothesize that molecular mechanisms like gene transcription and regulation, as opposed to genetic architecture (i.e., DNA sequence) or protein expression (i.e., receptor density/affinity), may be important for understanding differences in *ARB2* function between hypertensive and normotensive individuals.

Little is known about the genetic expression of ARs in human arterial tissue in the context of clinical HTN. Only one study to date has examined gene expression (RNA transcription) of AR between persons with and without HTN; however, the investigators used blood-based RNA detection and focused on the alpha-1A subtype (Veglio et al., 2001). It is generally accepted that receptors of any kind have different properties and expression levels in various tissue and cell types, making comparisons between blood and tissue studies suboptimal. It is also widely accepted that diseased tissue (rather than blood cells, lymphocytes, or mononuclear cells, for example) has the most potential for revealing disease mechanisms that are not primarily hematologic, largely due to the belief that the circulating transcriptome has the potential for interaction with many confounding substances and/or mediators within the blood, which is not a factor within tissue. For the aims of our study described here, arterial tissue is one appropriate source for studying HTN; however, tissue is the most difficult resource to safely and ethically obtain in human clinical research. As such, there is a knowledge gap regarding if and how *ARB2* mRNA is expressed in human arterial tissue in the context of clinical HTN.

Our primary aim in this study was to explore *ARB2* gene expression (mRNA levels) in the clinical phenotype of HTN by examining human internal mammary artery samples in coronary artery bypass patients with and without HTN. Human coronary artery contains equimolar amounts of ARB1 and ARB2 protein subtypes, with ARB2 specifically mediating vasodilatation in vascular smooth muscle types and, consequently, affecting BP (Monopoli et al., 1993). Ferro, Kaumann, and Brown (1993) reported predominantly ARB2-mediated relaxation specifically in human internal mammary artery exposed to both epinephrine and norepinephrine in vitro, providing a basis for harvesting this tissue type. We utilized gene expression techniques with human samples to address HTN in the real-world context of disease, emphasizing gene expression as a possible key to differences in ARB2 mechanisms in human HTN.

Material and Methods

Participants

We obtained Institutional Review Board (IRB) approval from the University of Florida and the Malcom Randall Veterans Administration (VA) Subcommittee for Clinical Investigations. English-speaking patients between the ages of 21 and 70 who were scheduled for coronary artery bypass graft (CABG) surgery involving the internal mammary artery (typical for bypass of the left anterior descending artery) were recruited by purposeful sampling. All participants gave written informed consent at least 1 day prior to their surgical intervention to allow them the opportunity to withdraw from the study (IRB

requirement). We enrolled 51 participants from north central Florida between August 2004 and July 2005. Surgical scheduling changes prevented collection of samples from 5 participants, while an additional 5 were excluded from analyses due to insufficient RNA yields and/or *undetermined* readings in the TaqMan real-time polymerase chain reaction (RT-PCR) gene expression detection system. The remaining 41 participants (17 normotensive and 24 hypertensive participants) were included in data analyses.

Study Design

This case-control study involved collection of normally discarded surgical remnants of internal mammary artery from patients undergoing scheduled CABG surgery. We preserved and stored the collected arterial remnants for batch analysis. Following tissue processing and RNA extraction, we performed TaqMan RT-PCR and used the delta delta cycle threshold (ΔΔCt) relative quantitation method for determination of fold-differences in gene expression between groups, normalized to glyceraldehyde phosphate dehydrogenase (*GAPDH*) gene expression levels. This analysis technique is an optimal exploratory starting point for detecting group differences; further investigations into mRNA copy number (discrete amounts of mRNA) and protein expression are typical next steps once differences are established with these methods.

Data Collection

Arterial tissue samples were collected in the surgical suites of one community and one VA hospital. The surgical remnants were cleaned of excess fasciae, sectioned, and placed in a microtubule of RNAlater™ solution (Qiagen, Valencia, CA). The samples were transported on ice to the UF Center for Pharmacogenomics Core Laboratory, where they were stored according to RNAlater™ instructions until batch analysis. Participants were grouped by diagnosis of HTN or normotension (NT) using the JNC VII criteria (Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, 2003). Participants were considered hypertensive if they were diagnosed by a practitioner (either by self-report or medical record documentation) or had medical-record documentation of three consecutive BP readings of 140/90 mm Hg or greater (with two of the instances at least 3 days apart). Participants were considered to be normotensive if both criteria were absent. Of the participants with documented normal BPs and no documentation of diagnosis of HTN, 7 were taking β-blockers (indicated for reduction of heart rate, treatment of arrhythmias, or to facilitate overall risk reduction in coronary heart disease, per treatment guidelines [Finnish Medical Society Duodecim, 2006]) and were placed into the NT group. Participants' β-blocker usage, type, and dose at the time of surgery were obtained from their medical charts. Positive family history of HTN was determined by asking participants to answer "yes" or "no" to having a first-degree blood relative (parent and/or sibling) diagnosed with HTN.

RNA Isolation and Reverse-Transcription

We treated all surfaces and tools with 0.1% solution of diethylpyrocarbonate (DEPC) to water for RNA laboratory work (Applied Biosystems, 2008). Tissues were removed from RNALater™ solution, gently blotted to remove excess solution, weighed, cut into smaller pieces, and transferred to a ceramic mortar. After adding liquid nitrogen, we ground the frozen samples into a fine powder with a ceramic pestle. We then combined the powdered tissue with 500 microliters of proprietary Lysis/Binding solution from the RNAqueous Kit (Ambion, Inc., Austin, TX) and homogenized the slush with a PowerGen 125 electric rotor-stator homogenizer (Fisher Scientific, Pittsburgh, PA). After a 30-s centrifugation to remove large debris, we removed the supernatant and extracted the RNA with the RNAqueous Kit, according to the manufacturer's instructions. We concentrated the seven samples that had concentrations of less than 10 ng/μL in a refrigerated Centrivap Console speed-vacuum (Labconco, Kansas City, MO). All RNA optic density 260/280 ratios were between 1.7 and 2.10. The quality of 18s and 28s peaks generated by a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) was consistent across all samples, indicating little RNA degradation. We reverse-transcribed samples with the cDNA Archive Kit (Applied Biosystems, Foster City, CA) per manufacturer's instructions and stored them at –20°C.

Real-Time Polymerase Chain Reaction

We prepared 20 μL reactions for singleplex RT-PCR with the ABI PRISM® 7900system (Applied Biosystems, Foster City, CA). Glyceraldehyde-phosphate

dehydrogenase (*GAPDH*) was used as the housekeeping, or normalizer, gene. We used the following ABI assays: *ARB2* #Hs00240532_s1 and *GAPDH* #4310884E (catalog number). For each reaction, we prepared 10.0 μL of TaqMan Universal PCR Master Mix (2X) with AmpErase UNG with 1.0 μL of each respective TaqMan Gene Expression Assay on Demand (20X). We singleplexed triplicate samples in the TaqMan system with PCR conditions of 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. We processed and analyzed fluorescence data with the ABI PRISM Sequence Detection Software (Applied Biosystems, Foster City, CA) and expressed results as cycle threshold, or Ct (number of cycles needed to generate a fluorescent signal above a predetermined threshold), as determined by the ABI 7900 software. The software determined the baseline automatically by assessing the normalized fluorescence signal versus cycle data per plate. From this baseline, we obtained each sample's Ct value.

Calculations for Relative Gene Expression and Selection of Calibrator

We imported all gene expression data into Microsoft Excel (Microsoft Corporation, Redmond, WA) for relative gene expression calculations. As the expected range of gene expression for the *ARB2* gene target in human internal mammary artery tissue is unreported, we considered all measured values valid; therefore, we removed no outliers. We used mean triplicate Ct values to calculate the following: (a) ΔCt = mean target Ct – mean endogenous control (*GAPDH*) Ct; (b) $\Delta\Delta Ct = \Delta Ct_{sample} - \Delta Ct_{calibrator}$; (c) $2^{-\Delta\Delta Ct}$, which is the normalized gene expression relative to the calibrator. The chosen calibrator was a normotensive, Caucasian male who was not taking any medications and reported no other cardiac or comorbid diagnoses. His body mass index (BMI) of 30.9 kg/m^2 was comparable to the sample mean BMI of 29.0 kg/m^2. He also reported not having ever been a smoker and did not drink or exercise.

The determination of fold-difference between groups is expressed as a ratio of the $2^{-\Delta\Delta Ct}$ medians for the groups compared. There is an inverse relationship between the Ct value and gene expression. This same principle applies even after normalization. All fold-difference data were calculated and interpreted in this fashion.

Statistical Analyses

We analyzed data using Microsoft Excel and SPSS version 14 (SPSS Inc., Chicago, IL). We used descriptive statistics to summarize demographic variables and we report normalized fold-differences (described previously) and significance values. To evaluate differences in gene expression of *ARB2* by diagnosis, we used the nonparametric Mann-Whitney *U* test. All hypotheses were two-tailed. We set α at .05 for the first statistical comparison by diagnosis and Bonferroni corrected additional tests setting α at .008. We categorized the β-blocker variable by dose for metoprolol tartrate (refer to Table 1). One participant was taking Labetalol 300 mg/day. This dose was found to be equivalent to 100–200 mg/day of metoprolol (Lacy, Armstrong, Golman, & Lance, 2005); therefore, we placed this participant in the "greater than 50 mg twice daily" category for analyses. To evaluate the efficacy of the housekeeping gene, *GAPDH*, we performed nonparametric Spearman's rho to test correlations between average triplicate Ct values for *GAPDH* and the *ARB2* targets. In addition, we used Student's *t*-test to evaluate variability of the normally distributed *GAPDH* expression by diagnosis.

Results

Participants

Table 1 shows a summary of the demographic data. We report gene expression data from 17 (41.5%) normotensive and 24 (58.5%) hypertensive participants whose mean age was 57.1 years. A majority of our participants were Caucasian, non-Hispanic males. Hypertensive and normotensive participants did not significantly differ by age, race, anthropomorphic variables, family history, or type of surgery facility (Table 1). Collectively, the demographic data suggest that this sample is moderately representative of the population of bypass patients who undergo surgery in university hospital and Veterans Administration settings (i.e., primarily Caucasian males in their midfifties, overweight or obese [mean BMI of at least 29], most with HTN and diabetes); however, it may not be representative of the entire population of people with coronary artery disease or essential hypertension.

ARB2 Gene Expression by Diagnosis

Data are expressed as a ratio of the $2^{-\Delta\Delta Ct}$ medians for the groups compared. Median $2^{-\Delta\Delta Ct}$ and

Table 1. Participant Demographics

Parameter	Normotensive Group, $n = 17$	Hypertensive Group, $n = 24$	p^a
Age (years), mean (*SD*)	56.7 (7.3)	57.5 (7.9)	.43
Women, *n* (%)	2 (11.8)	7 (29.2)	.21
Race, *n* (%)			–
White/Caucasian	14 (82.5)	15 (62.5)	
Black/African American	2 (11.8)	7 (29.2)	
White/Caucasian & Native American	1 (5.9)	2 (8.3)	
Hispanic ethnicity, *n* (%)	0	1 (4.2)	–
Height (m), mean (*SD*)	1.77 (0.10)	1.73 (.114)	.18
Weight (kg), mean (*SD*)	87.5 (17.73)	89.68 (19.92)	.65
BMI (kg/m^2), mean (*SD*)	27.91 (5.09)	30.16 (6.18)	.14
β-blocker, type & dose, *n* (%)			–
Not prescribed	10 (58.8)	6 (25.0)	
Metoprolol tartrate, 12.5 mg BID	2 (11.8)	4 (16.7)	
Metoprolol tartrate, 25 mg–50 mg BID	4 (23.5)	11 (45.8)	
Metoprolol tartrate[b] > 50 mg BID	0 (0)	3 (12.5)	
Missing data	1 (5.9)	0 (0)	
Type 2 diabetes mellitus, *n* (%)	0	13 (54.2)	[c]
Family history of HTN, *n* (%)			.32
Yes	7 (41.2)	13 (54.2)	
No	5 (29.4)	4 (16.7)	
Missing	5 (29.4)	7 (29.2)	
Surgery facility, *n* (%)			.67
Community hospital	12 (70.6)	20 (83.3)	
Veterans Administration hospital	5 (29.4)	4 (16.7)	

Notes: BID = twice per day; BMI = body mass index.
[a] *p* Values are for tests of significant differences of characteristic between normotensive (NT) and hypertensive (HTN) groups.
[b] Or dosing equivalent of labetalol.
[c] Test of significance not performed due to absence of type 2 diabetes mellitus cases in NT group.

Table 2. Median $2^{-\Delta\Delta Ct}$ and Standard Deviation Values by Cohort

	Diagnosis[a]	
Cohort	HTN	NT
All participants	(24); 0.69 ± 9.76	(17); 0.25 ± 4.49
Caucasians	(17); 1.02 ± 11.25	(15); 0.21 ± 4.8
African Americans	(7); 0.16 ± 4.49	(2); 1.14 ± 0.91

Notes: a. HTN = hypertension group; NT = normotension group.
[a] Group sample sizes in parentheses.

standard deviation values are presented in Table 2. *ARB2* gene expression was significantly lower in hypertensive participants ($n = 24$) compared to normotensive participants ($n = 17$; 2.76-fold, $p = .035$). The 2.76-fold difference was determined by a ratio of hypertensive to normotensive $2^{-\Delta\Delta Ct}$ values (0.69/0.25). The higher $2^{-\Delta\Delta Ct}$ values for hypertensive participants indicates a lower level of gene expression because it took more TaqMan amplification cycles to detect the mRNA (i.e., there was less mRNA present) than for the normotensive participants. Though the data could also be interpreted as normotensive participants having 0.36-fold higher gene expression level than hypertensive participants (0.25/0.69), we chose to interpret the data in the former fashion as we are interested in the expression level in HTN as it compares to NT.

Models of cardiovascular reactivity (Anderson, McNeilly, & Myers, 1992), studies of pharmacologic response (Jennings & Parsons, 1976), and gene-association studies (Wood, 2002) support differential adrenergically mediated processes for HTN in different racial cohorts. For this reason, we removed self-reported African American participants from the analysis and determined gene expression differences in Caucasians. In this analysis, participants who self-reported both Caucasian and Native American ($n = 3$) were combined with the White/Caucasian group. As shown in Table 2, *ARB2* gene expression was significantly lower in hypertensive Caucasians ($n = 17$) compared with normotensive Caucasians ($n = 15$; 4.86-fold, $p = .008$, Bonferroni corrected).

Post Hoc Analyses for Reclassified Diagnosis

To determine if the 7 normotensive participants who were taking β-blockers had any effect on the analyses, we removed these participants from the normotensive group and repeated the aforementioned analyses. There was only one change in the significance of findings as a result of this action: the fold-difference in *ARB2* gene expression between Caucasian HTN and Caucasian NT groups was no longer significant after Bonferroni correction ($p = .02$); however, it remained statistically significant in the comparison of all normotensive participants with all hypertensive participants ($p < .008$). The shortcoming of this approach is that removing 7 participants from the normotensive group reduces the power, likely explaining the change in significance for the one test. However, the outcome from this post hoc analysis provides some evidence that β-blocker usage in the NT group did not seriously deteriorate the construct validity of the NT classification variable.

ARB2 and Covariates

Having access to valuable clinical data on the participants' medical and personal history allowed us to compare diagnosis groups' target expression differences by gender, diagnosis of type 2 diabetes mellitus, and positive family history of HTN, with the goal of evaluating potential for confounding. With further Bonferroni correction for multiple tests, no significant differences were found in target genes' expression for these variables (data not shown). Of note, confounding by β-blocker use was theoretically not expected, as the β-blocker usage for our participants was limited to ARB1-acting medications. It has been reported that metoprolol treatment increases ARB1 but not ARB2 protein expression in the human heart (Brodde, Daul, & Michel, 1990). In our data, β-blocker usage was not a significant confounder statistically (data not shown). The lack of significant findings may indicate that these are not confounding variables with regard to gene expression; however, the limitation of reduced power and the conservatism of Bonferroni correction also need to be considered.

Normalization With *GAPDH*

Review of the literature revealed support for the use of *GAPDH* as a normalizer in similar tissue types (Wang & Brown, 2001; Peuster, Fink, Reckers, Beerbaum, & von Schnakenburg, 2004). The triplicate *GAPDH* Ct values had a mean and standard deviation of 28.27 $\pm$ 2.99 and median of 28.23 with a range of 22.24−35.33. *GAPDH* gene expression (average triplicate Ct) was not correlated with *ARB2* gene expression ($R = 0.23$, $p = .142$) but was significantly correlated with diagnosis ($t = -2.61$, $df = 39$, $p < .05$).

Discussion

Our data provide preliminary support for a possible molecular mechanism contributing to impaired vasorelaxation in HTN via downregulation of *ARB2* gene expression. We have been unable to find other reports of human arterial tissue *ARB2* gene expression for true comparison of findings. However, gene-delivery research has provided findings that are consistent with *ARB2* gene dysregulation in HTN. Gaballa and colleagues (1998) reported enhanced ARB2-mediated vasorelaxation in de-endothelialized rat carotid arteries after adenovirally mediated delivery of the *ARB2* gene. Similarly, overexpression of *ARB2* in systemic hypertensive rats and (normotensive) Wistar-Kyoto rats enhanced ARB2-mediated vasorelaxation in both rat strains, with a lessened vasodilatory effect in the hypertensive strain (Iaccarino et al., 2002). The authors concluded that this vasorelaxation was directly related to ARB2 signaling dysfunction and not endothelium-dependent nitric oxide metabolism. Our data support a potential molecular mechanism for these findings. Based on our results, we infer that a contributing mechanism for HTN could be reduced vasodilation due to the *ARB2* gene being downregulated.

To take our findings one step further, we would also expect to see reductions in ARB2 protein levels in HTN. Brodde et al. (1985) report, however, that protein expression of ARB2 was increased in their study of HTN. Some possible explanations involve the fact that the AR system is part of a physiologic feedback loop. The timing of our measurement of the *ARB2* mRNA (and similarly, the timing of Brodde and colleague's ARB2 protein measurements) within the negative feedback system makes interpretations difficult. First, the Ct values obtained with TaqMan RT-PCR represent only steady-state mRNA levels (Bustin, 2002), limiting our interpretations of the suspected effects of these transcription levels on

posttranscriptional processes. We could infer that the system is downregulating *ARB2* gene expression because ample (increased) ARB2 protein has already been produced to thwart the increase in BP. Additionally, the increased ARB2 protein expression seen by Brodde et al. could imply that the body is attempting to invoke vasodilation via the ARB2 mechanism because of the hypertensive (vasoconstrictive) state where there was already impairment in *ARB2* gene expression. The complexity of the negative feedback loop in the neurohumoral model of HTN thus makes it difficult to determine cause and effect but does provide an explanation for how these two different results (decreased gene expression but increased protein expression) can coexist. Furthermore, our investigation and the work of Brodde and colleagues use different cell types, which are not comparable. Additionally, there is compelling evidence that lymphocyte expression of G-protein-coupled receptor kinase 2 (GRK2; also known as beta adrenergic receptor kinase, or BARK) may be responsible for the impairment of *ARB2* responsiveness in human (Gros, Benovic, Tan, & Feldman, 1997; Gros et al., 1999) and animal (Gros et al, 2000) forms of HTN. Targeting *ARB2* desensitizes the receptor to overstimulation by epinephrine (Pippig et al., 1993; Pitcher, Lohse, Codina, Caron, & Lefkowitz, 1992). These data suggest that mediators other than *ARB2* mRNA alone can affect the ARB2 signaling complex and lead to impaired vasodilation.

These points highlight the complexity of the biological and physiological influences on HTN as well as the difficulty of accurately capturing meaningful data in a constantly changing physiological system. The impact of gene expression in this complex system cannot be truly established unless future studies measure mRNA and protein at the same time points from the same biological source and account for the other cofactors involved in signaling of ARB2. As none of the studies discussed are sufficient to explain cause and effect and used different species, methods, and comparisons, interpretations are necessarily limited.

As expected, group differences in *ARB2* gene expression remained significant when comparing Caucasian participants by diagnosis, as they made up a majority of the sample. This finding is consistent with prior observations of impaired ARB2-mediated vasodilation in Caucasians (Anderson, McNeilly, & Meyers 1992; Naslund, Silberstein, Merrell, Nadeau, & Wood, 1990).

Regarding expression normalization, *GAPDH* gene expression was not significantly correlated with expression of the *ARB2* gene (a desired result indicating normalization with this gene is appropriate). The significant correlation of *GAPDH* gene expression with diagnosis of HTN weakens our confidence in *GAPDH* being invariably expressed among all samples. The literature regarding normalization with *GAPDH* is varied; its use for normalization with human arterial tissue has not been widely studied. Our group consulted with Ambion representatives, in addition to the literature, which led to our choice of *GAPDH* as the housekeeping/reference gene for this study. The significant correlation we found between *GAPDH* and HTN limits our study. For future investigations of this nature, we acknowledge the recommended use of multiple normalization genes (Vandesompele et al., 2002) or endogenous control gene panels for thorough assessment of normalization genes' performance in the tissue of interest.

Table 3. Effect Sizes and Numbers Needed to Power Future Studies

Analysis	Effect Size	Number Needed[a]
All participants by diagnosis[b]	0.35	72
Caucasians by diagnosis[b]	0.40	64
African Americans by diagnosis	0.27	100

[a] The number of participants needed in each group to achieve a power of 0.80. Assumes equal numbers in each group.
[b] Significant finding in current analyses.

Limitations

There were a number of limitations in this investigation. We had limited power to detect differences between groups. We performed an *a posteriori* power analysis to generate Cohen's d effect sizes for our aims. These values were then used to calculate the number of participants needed in each group to achieve 80% power (see Table 3). This table may be useful to others interested in determining sample and effect sizes for similar work. Noninclusion of emergent bypass cases creates a sampling bias. Another potential limitation concerns construct validity of the "normotension" diagnosis, as some participants taking antihypertension medications for indications other than HTN were considered normotensive. Other medications used for treatment of HTN (diuretics, ACE inhibitors, and calcium channel blockers) may have been prescribed for non-HTN indications

like heart failure, diabetic nephropathy, or peripheral vascular disease. In determining diagnosis status for this study, participants were categorized as hypertensive based on practitioner diagnosis and/or actual BP values that met the JNC VII criteria. Additionally, those considered "normotensive" at the time of the study could develop HTN in the future; thus, their biophysiologic mechanisms could be different from those who will never develop HTN, and likely represent a different phenotype of preclinical HTN. Finally, at the outset of designing this study, we anticipated the potential confounding of transcription levels by the presence of pharmacologic β-blockade and the diagnosis of type 2 diabetes (Bengtsson et al., 2001), however, our data do not support this.

Conclusions

Genetics and genomics hold promise as means by which to tease out the pathobiological mechanisms and risk factors contributing to the widespread disease of HTN. With the present study, we demonstrated that our human-tissue collection methodology was feasible and shows potential for use in future research. The *ARB2* gene had lower expression in hypertensive than normotensive participants by almost threefold, a significant difference that supports a potential molecular basis for *ARB2* dysregulation in the neurohumoral model of HTN. The significance of this finding is tempered by the normalization issues discussed above. Other factors not studied (for example, transcription factors, AR protein levels, or interactions with regulatory regions and other proteins) may contribute to expression differences in the *ARB2* gene and should be studied in the future. Because of the limited amount of harvested tissue and the exploratory nature of this study, we did not aim to determine ARB2 protein levels. It is currently accepted, however, that mRNA levels do not correlate with subsequent protein levels, despite mRNA's primary role in translating proteins. Despite lack of protein data for functional correlation, these data can inform whether or not mRNA levels differ by disease state in the actual tissue.

Research of this nature—using human samples in the context of clinical phenotypes—is imperative to future translational studies to refine our understanding of genetic mechanisms of complex disease and identify risk factors with a significant genetic component. Modified replication of this research is needed to validate findings. Modifications should include further optimizing normalization in human arterial tissue and minimizing the impact of potential confounders.

Overall, this approach can be informative of disease mechanisms at the genomic level, providing information that can lead to translation of clinical detection for patient-specific genomic markers in the future. This method is not likely to directly influence practice, as removing arterial tissue during bypass surgery and analyzing gene expression in this fashion would not be amenable to the current health care setting. Rather, investigating gene expression within tissue (and possibly blood) may provide sound support for molecular-based alterations in the hypertensive disease process.

BP measurements, alone, do not paint a complete picture of the hypertensive mechanism for a particular individual and, as such, treatment modalities are often a trial-and-error approach to reducing and maintaining BP. The state of the science is moving toward personalized medicine in which clinicians will be able to tailor treatments to improve results and reduce side-effects and drug interactions. If gene-expression impairments for HTN are detectable and reproducible in the clinical population, future screening and treatment for HTN could be positively affected. The practice of screening could be modified to add molecular screening, which is likely to be more informative than BP and risk factors alone. Extension of this research into more detailed transcriptional and posttranscriptional mechanisms could potentially support gene therapy involving *ARB2* gene mechanisms in human populations. Further research could elucidate how environmental variables affect *ARB2* gene expression, how such factors correlate with BP phenotypes, and how *ARB2* gene expression responds to different treatment modalities.

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