Association of a Multi-Gene Panel with Blood Pressure Medication Success in Patients with Hypertension: A Pilot Study

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Running Title: Multi-Gene Panel and Blood Pressure Response in Hypertension

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Abstract:

Several common and functional genes are known to contribute to responsiveness to blood pressure (BP) therapy. BP therapy is typically guided by algorithms that do not include a patient's genetic information. This study aimed to determine the impact of a multi-organ genetic panel on BP response to pharmacotherapy. Eighty-six patients completed one study visit consisting of a buccal swab collection, measurement of office BP, and a medical chart review for BP history. Genes analyzed included those that encode for one drug metabolizing enzyme, renal Na^+ handling, vascular, and cardiac function. Relationships between genotype and control of BP (<140/<90), Δ systolic BP, Δ diastolic BP, and Δ mean arterial BP were assessed. SLC12A3 resulted in a significant association between the target drug and the functional genotype for BP control (<140/<90 cut off) ($p<0.05$). Conversely, three of five renal genotypes were associated with BP control using 120/80 as a cut-off ($p<0.05$). Three of four cardiac genotypes were associated with the BP control at <140/<90, with one being statistically significant (position 49 of ADRB1). Only one vascular genotype was predictive of blood pressure control at <140/<90. We found a significant drop in mean BP from baseline in six genes, three important in the diuretic response and three in β-blockade ($p<0.05$ on target drug vs. not). These results demonstrate that a multi-gene panel for renal Na^+ handling, vascular function, and cardiac output may influence the BP response to therapy, but larger studies with more statistical power are needed.

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Keywords: Hypertension; Blood pressure; Genetics; Pharmacogenetics; Pharmacotherapy; Treatment

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Introduction:

Hypertension (HTN) is one of the most important preventable contributors to disease and death in the United States, and represents the most common condition seen in the primary care setting[1,2]. Hypertension affects approximately 80 million individuals in the United States, with more than 5 million new diagnoses made each year[3,4]. High blood pressure (BP) is responsible for ~360,000 deaths annually and, in 2009, had a direct cost to the United States healthcare system of approximately $51 billion dollars[5,6]. Rapid reductions in BP are important for survival in hypertensive patients, as end organ damage occurs quickly, and even small (~5 mmHg) reductions in BP can dramatically improve survival[7]. Hypertension control rates are generally poor, with approximately 50% of patients eventually reaching control[8]. This level of control goes well beyond adherence rates, since 40% of patients who take their medication as prescribed do not have their BP under control[9]. Further, each common class of BP medication (diuretic, ACE-inhibitors, angiotensin-II receptor blockers) has an average effectiveness rate of 50%, suggesting that a genetic component plays a role in therapy efficacy[10]. Contrary to conventional wisdom and clinical appreciation, there is a bell-curve response to most hypertension therapies, such that a proportion of patients have a reduction in BP, but 10-20% of patients have no change in BP, or even an increase in BP[11,12]. Despite this lack of general effectiveness for BP response to therapy, the current standard of care is to “layer” BP drug therapies in an effort to control HTN. Unfortunately, this layering approach has potential consequences in the form of increased side effect profile, costs to the patient, increased health care service utilization, and reduced quality of life[13,14].

Like many diseases, there is a heritable component to the development of HTN, which is estimated to be around 50%, with emerging data suggesting that treatment for HTN may be

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heritable as well[12,15-18]. The risk of developing HTN doubles for each first degree relative with HTN, and sons of HTN patients have an average of 10 mmHg higher systolic and diastolic BP when compared to sons of normotensive individuals, independent of Na^+ intake[15,19]. Further, there is a 55% correlation for susceptibility to HTN in monozygous twin siblings, up to 40% correlation in dizygous twins, and as low as 20% for non-twin sibling pairs[20-22]. Collectively, these data clearly demonstrate a heritable component to the development of HTN. The heritability of the treatment for HTN is less clear. While it is clear that hypertension response rates improve when genetic scoring is used to guide therapy[17], and monotherapy responsiveness can be improved using genetics[16], few studies have explored the impact of genotype on multiple drug classes simultaneously[11,17]. Much of the previous work to determine the genetics of HTN therapy initially focused on genome-wide association studies followed by elegant studies of response rates to thiazide diuretics, β -blockers, and ACE-inhibitors, but each has typically been done in isolation (e.g. genotype for renal genes to determine the response to a thiazide diuretic within one study)[11,12,23-25].

While the promise of using genetic information to guide medical therapy existed before the human genome was fully sequenced, there is a general lack of genetically informed therapy decisions in clinical practice today, particularly across multiple classes of drugs for a given disease. Currently, no validated genetic panel has previously been studied to guide HTN therapy. In contrast, and in support of precision medicine for HTN, use of an individual's plasma renin levels can improve BP control by approximately 40%[26]. This highlights the importance of using an individual's own clinical information to guide therapy. One shining star example of the use of precision medicine to guide (and even develop new) therapies is in genotyping for the cystic fibrosis transmembrane conductance regulator (CFTR) in patients with cystic fibrosis

 doi:10.20944/preprints201810.0028.v1

(CF). In this model, patients are prescribed specific medications that are more effective according to their CFTR genotype status[27-29]. Although CF is a monogenic disease, there are over 1700 genotypes of CFTR which lead to distinct phenotypes within this disease, resulting in a heterogeneous disease with distinct pathways for treatment. This model of pharmacogenetics in CF demonstrates an appreciation for: a) the functionality of a protein (or channel), b) how genetic variation can influence that protein, and c) how drug therapy can be altered to match that genetic variation. Very few studies have utilized this type of approach outside of CF because of pathways that are more complex and the integrative nature of diseases like hypertension.

Given the impetus for using genetics in understanding the risk of development of HTN, coupled with the generally poor rates of control using the current standard of care, we sought to determine the association between a multi-gene panel on BP response to HTN therapy in patients with controlled HTN (<140/<90). We hypothesized that polymorphisms contained in genes responsible for determining functionality of proteins within specific organ systems involved in BP regulation (kidney, heart, and vasculature) would be associated with the effectiveness of a patient's BP therapy.

Methods:

Study design:

This pilot study was designed and performed as a retrospective study of patients with HTN, but included only patients who had a history of BP control (<140/<90) (clinicaltrials.gov identifier: NCT02524873). The study involved one clinic visit which consisted of collection of a buccal swab, measurement of office BP, and completion of a medication history survey. The study visit was followed by a thorough clinical chart review of the patient's HTN and medication history.

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The primary outcome variable for analysis was office BP at time of buccal cell collection. From this BP measurement, we were able to assess the level of BP control (percent of patients who had a BP that was <140/<90) and the change in BP (with the change in BP defined as the difference in BP from diagnosis to the office visit of the present study). From the chart review and the office visit, we were able to assess the number of medications and office visits needed to attain BP control, from the time of the initiation of therapy, the HTN medication side effect profile, as well as HTN associated adverse events during the course of treatment. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by Clinicaltrials.gov (identifier: NCT02524873).

Subjects:

All patients enrolled in the study were HTN patients who had their BP under control and who had been diagnosed with HTN for at least one year. Patients provided written informed consent prior to enrollment and data collection (Chesapeake IRB# 00011237). In addition to BP history, demographic information collected included age, sex, height, weight, and self-identified race, and ethnicity. Inclusion criteria for the study included: 20-85 years of age, patient on the same class/classes of BP medication for a minimum of six months (a change in dosage, frequency, or specific medication was accepted as long as there have been no changes to the class/classes of medications prescribed), a body mass index (BMI) between 19 and 45kg/m^2, patient had to be prescribed and taking one of the following classes of medications alone or in combination: diuretics (thiazide or thiazide-like), ACE inhibitors, angiotensin receptor blocker (ARB), β-blockers. Subjects were excluded from participation in the research study if one or more of the following conditions were met: a diagnosis of secondary HTN or a complication of

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pregnancy, currently prescribed and taking any additional class of medication(s) for high BP not included in the inclusion criteria list, or systolic BP > 190 or diastolic BP > 120 documented within the immediate six months prior to the study visit.

Cell collection and genotyping:

For this study, we collected two buccal swabs. The patient first collected cells via a buccal brush by swabbing the inside of their right cheek repeatedly (for five seconds using moderate pressure) (A-swab). The patient then deposited the swab in 750ul of lysis buffer consisting of 50mM Tris pH 8.0, 50mM EDTA, 25mM Sucrose, 100mM NaCl and 1% SDS to lysethe cells and stabilize DNA during transit prior to extraction. This process was repeated with the left cheek (B-swab) to ensure adequate cell collection necessary to achieve a minimum yield of 500ng total gDNA necessary for downstream genotyping. Subsequent lysate from buccal swabs was used in DNA isolation via Qiagen DNeasy isolation kits according to manufacture recommended specification (Qiagen). Patient isolated DNA was then assayed for, 14 functional alleles in 11 genes selected for known functionality in the heart, kidney and vasculature from previous peer-reviewed studies: (2) SNPs in ADRB1 (rs1801252 and rs1801253), (2) SNPS in ADRB2 (rs1042713 and rs1042714), SCNN1A (rs2228576), alpha-adducin (ADD1, rs4961), SLC12A3 (rs1529927), (2) in WNK1 (rs1159744 and rs2107614), angiotensin-converting enzyme (ACE, rs1799752), angiotensin (AGT, rs699), angiotensin receptor (AGTR1, rs5186), cytochrome P450 2D6 (CYP2D6*4, rs3892097), and renin (REN, rs12750834). With the exception of the ACE insertion/deletion (indel) genotype, all genotype polymorphisms were quantified using a two-step process beginning with a multiplex PCR as indicated in Supplemental Table 1, directly followed by a single base extension (SBE) reaction provided in Supplemental Table 2. The products of the SBE reaction were pooled and subsequently flown on

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a genomic mass spectrometer (Agena MassARRAY system) to generate individual genotypes. The ACE indel status was assessed using a standard PCR primer set and protocol provided in Supplemental Table 3, followed by 2% agarose sizing gel electrophoresis. Pre-characterized Coriell cell line DNAs consisting of all possible genotype combinations were run in parallel for each SNP as controls. All genotype data in aggregate was also used to compute population allele frequencies which were then confirmed against known existing frequencies in publically available databases (ExAC browser, 1000 Genomes project, GO-exome sequencing project, and TOPMED.

Data analysis:

All data were coded for statistical analysis (i.e. drug classes and genotypes coded numerically according to functionality) and were analyzed with SPSS v.21. Normality of the data were assessed using Levene's test prior to statistical analysis to assess equality of variance and correct statistical tests accordingly. Descriptive statistics were computed (average time for BP control, average number of visits to the clinician for BP control, age, height, weight, BMI, etc.). Post-hoc correction for univariate analysis of variance was conducted using Bonferroni analysis. Ordinary least squares regression via univariate modelling was used to estimate the magnitude of linearity between drug classes that yielded the best BP control and genetic profile of the subject. All statistical analyses were considered significant at an alpha level of 0.05. All data are reported as mean ± SD.

Results:

Eighty-six patients completed all aspects of the study and were included in the statistical analysis (subject characteristics, table 1). Upon diagnosis with HTN, the mean BP was 151/91 mmHg and the final office visit BP was 134/82 mmHg (table 2). The patients were on an average of 1.8 BP medications, with the most common

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medication being an ACE inhibitor and the least common medication being a Ca^+ channel blocker (table 3). There was considerable variability in the time to control (in months) and the number of clinic visits before BP control was attained according to drug class (table 3). On average, it took approximately one year to attain BP control, with the quickest time to control being for a Ca^+ channel blocker (although this required two additional clinic visits, when compared to an ACE-inhibitor therapy). The average number of clinic visits needed to attain BP control was four, which also varied according to drug class.

Table 1. Patient demographics**.**

Age (yrs)	58±0.8
Sex (% female)	46
Diabetes (% with)	28±4
Weight (kg)	86±1.4
Height (cm)	169±1
BMI (kg/m^2)	29.9±0.4
Number of Classes of Drugs for HTN	1.8±0.08

BMI=body mass index (kg/m^2).

Table 2. Blood pressure response to treatment.

Baseline Blood Pressure	
SBP (mmHg)	151±2
DBP (mmHg)	91±1
MAP (mmHg)	111±1
Nadir of Blood Pressure	
Lowest SBP in past two years (mmHg)	115±1
Lowest DBP in past two years (mmHg)	72±1
Current Blood Pressure	
Current SBP (mmHg)	134±2
Current DBP (mmHg)	82±1
Current MAP (mmHg)	99±1
Time to BP control (months)	22±10
Clinic Visits in the Past two years for HTN	3.6±0.3

SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial blood pressure.

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Table 3. Time to control according to blood pressure therapy class.

		Months For Control		Clinic Visits/2Years	
Drug Class	**On the Drug Class (%)**	**On the Drug Class**	**Not on the Drug Class**	**On the Drug Class**	**Not on the Drug Class**
Diuretic	42±5	19.5±20.4	7.9±4.2	4.5±0.6	3.0±0.4*
ACE Inhibitor	62±5	22.2±11.4	22.5±16.4	3.1±0.4	4.5±0.6*
Antiotensin Receptor Blocker	27±5	22.8±23.1	17.1±9.1	3.9±0.6	3.5±0.4
B-Blocker	33±5	24.5±16.9	21.2±12.0	4.9±0.7	3.1±0.4*
Ca^+ Channel Blocker	16±4	9.9±4.5	25.0±11.7	5.1±0.7	3.3±0.4

ACE=angiotensin converting enzyme, angiotensin receptor blocker.

Genotype distribution is presented in table 4. The response to the level of BP control with target therapy according to published functional genotypes yielded variable results which seemed to improve with more strict limits set for BP control (<120/<80, but this also resulted in a smaller sample size for analysis) (table 5). Only one of the alleles in the genes associated with renal activity (meaning renal Na^+ handling, SLC12A3) resulted in a significant association between the renal targeted drug, diuretic, and the functional genotype for BP control when using <140/<90 as a cut off ($P<0.05$). In contrast, three out of five functional genotypes involved in renal Na^+ handling (WNK, SLC12A3, and SCNN1A) were associated with BP control using the more strict 120/80 as a cut-off ($p<0.05$). While the general functionality of all but one of the functional alleles in genes associated with cardiac function were also associated with the BP control at the <140/<90 level, only one of these was statistically significant (position 49 of the ADRB1, $p<0.05$). In contrast to the genotypes linked to renal Na^+ handling, there was no effect of the cardiac-linked genotypes in percent of patients under control at the more strict level of BP control (<120/<80). Similar to the renal genotypes, within the vascular genotypes only one genotype was statistically predictive of BP control at the <140/<90 level (ANG) ($P<0.05$), while none were statistically associated with control at the stricter cut-off level.

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Table 4. Genotype distribution.

Renal Genes	**rs1159744**	**WNK1a**	CC(10)	CG(29)	GG(42)
	rs2107614	**WNK1b**	CC(33)	CT(36)	TT(20)
	rs1529927	**SLC12A3**	CC(1)	CG(7)	GG(77)
	rs2228576	**SCNN1A**	CC(41)	CT(31)	TT(10)
	rs4961	**Alpha Adducin**	GG(63)	GT(21)	TT(5)
Cardiac Genes	**rs1042713**	**ADRB2 16**	CC(7)	CG(41)	GG(28)
	rs1042714	**ADRB2 27**	CC(26)	CG(46)	GG(10)
	rs1801252	**ADRB1 49**	AA(27)	AG(36)	GG(71)
	rs1801253	**ADRB1 389**	CC(43)	CG(30)	GG(8)
	rs3892097	**CYP2D6**	CC(56)	CT(22)	TT(11)
Vascular Genes	**rs12750834**	**Renin**	AA(4)	AG(20)	GG(66)
	rs699	**Angiotensin**	CC(19)	CT(41)	TT(29)
	rs5186	**Antiotensin-2 Receptor**	AA(49)	AC(30)	CC(9)
	rs1799752	**ACE**	II(15)	ID(45)	DD(29)

Genotype (count)

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Table 5. Percent of patients with a functional genotype who have blood pressure under control taking the target drug class vs. not on target drug class.

			n	<140/<90	<120/<80
Renal Genes	**rs1159744**	**WNK1a**			
		Not On Diuretic	15	66±10	4±4
		On Diuretic	24	53±13	33±12*
	rs2107614	**WNK1b**			
		Not On Diuretic	40	67±8	7±4
		On Diuretic	27	56±10	22±8*
	rs1529927	**SLC12A3**			
		Not On Diuretic	49	66±8	16±16
		On Diuretic	35	100±0*	50±50
	rs2228576	**SCNN1A**			
		Not On Diuretic	20	70±11	10±6
		On Diuretic	20	45±12	25±10
	rs4961	**Alpha Adducin**			
		Not On Diuretic	11	80±13	0±0
		On Diuretic	11	46±16	18±12*
Cardiac Genes	**rs1042713**	**ADRB2 16**			
		Not on B-Blocker	51	53±7	15±5
		On B-Blocker	26	62±9	11±6
	rs1042714	**ADRB2 27**			
		Not on B-Blocker	48	54±7	14±5
		On B-Blocker	23	65±10	8±6
	rs1801252	**ADRB1 49**			
		Not on B-Blocker	55	51±6	14±5
		On B-Blocker	27	67±9*	11±6
	rs1801253	**ADRB1 389**			
		Not on B-Blocker	21	67±10	17±7
		On B-Blocker	6	50±8	22±8
	rs3892097	**CYP2D6**			
		Not on B-Blocker	22	66±21	14±14
		On B-Blocker	6	73±9	27±10
Vascular Genes	**rs12750834**	**Renin**			
		Not On Angiotensin Receptor Blocker	17	58±12	11±8
		On Angiotensin Receptor Blocker	7	43±20	28±18
	rs699	**Angiotensin**			
		Not On Angiotensin Receptor Blocker	17	41±12*	16±5
		On Angiotensin Receptor Blocker	50	60±7	18±10
	rs5186	**Antiotensin-2 Receptor**			
		Not On Angiotensin Receptor Blocker	10	60±16	18±12
		On Angiotensin Receptor Blocker	28	71±8	18±7
	rs1799752	**ACE**			
		Not on ACE Inhibitor	10	65±10	15±7
		On ACE Inhibitor	28	49±7	15±5

One limitation with assessing the percent of patients to attain BP control is that this does not account for a patient's starting point for their BP treatment upon diagnosis. When this is taken into account (through the assessment of mean change in BP) we demonstrate a statistically significant effect in more than half of the genotypes and a clinically meaningful (>4mmHg difference between on the target therapy vs. not) and statistically

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significant effect in six of the target genes, even in this small retrospective trial, three important in the diuretic response and three important in β-blockade (WNK rs1159744, SLC12A3, alpha-adducin, ADRB2, and CYP2D6)(P<0.05 on target drug vs. not)(table 6). Therefore, when combined, we find that a majority of the genes in the present study result in differential functionality when looking at both BP control (<140<90) and delta BP. These data help to explain the dramatic variability with respect to the percent of patients under control according to genetic variation within the renal, vascular, and cardiac systems.

Table 6. Mean blood pressure change in genotypes that demonstrated a statistically significant change in blood pressure.

	Not on Target Drug	On Target Drug
Diuretic		
rs1159744		
SBP	-14.4±4.1	-20.5±6.3*
DBP	-5.9±2.2	-12.3±3.7*
MAP	-8.6±2.4	-14.8±4.2*
rs1529927		
SBP	-35.0±7	-29.5±-35
DBP	-3.5±6.7	-13.1±13*
MAP	-8.1±6.9	-18±20*
rs4961		
SBP	-6.6±6.6	-15.3±6.2*
DBP	-7.9±3.6	-9.9±4.3
MAP	-7.3±3.7	-11.5±4.5*
B-Blocker		
rs1801252		
SBP	-12.2±2.9	-22.0±3.6*
DBP	-6.1±1.6	-11±2.6
MAP	-7.9±1.8	-14.5±2.7
rs1042713		
SBP	-14.7±2.9	-22.3±3.8
DBP	-6.5±1.6	-11.0±2.7
MAP	-9.0±1.8	-14.5±2.6*
rs3892097		
SBP	-16.9±3.7	-24.0±7.5*
DBP	-7.7±2.7	-6.6±6.2
MAP	-10.6±2.6	-12.2±6.1

*p<0.05 when compared to patients not on the target drug for that gene.

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Discussion:

In this pilot study, we assessed the relationship between known, common, and functional gene polymorphisms responsible for encoding proteins within the renal, vascular, and cardiac organ systems on the BP response to HTN therapies in patients with HTN. Interestingly, very little data exists on the time to BP control in patients with HTN and the number of clinic visits needed to attain BP control within the US healthcare system. In the present study, we show that HTN is controlled, on average, one year after initial diagnosis, with an average of four clinic visits. Considering the magnitude of downstream consequences of HTN and the economic burden HTN has on the healthcare system, it is imperative to improve this time to control. In addition, we found that more than half of the genotypes selected for our multi-organ system analysis influenced either BP control (<140/<90) or a delta BP (systolic, diastolic, or mean arterial). It is likely that using these common genotypes to guide therapy will improve a patient's time to control, decrease the number of medications that a patient is taking, and improve medication adherence (through improved effectiveness and decreasing the number of medications used). While a great deal of literature exists on the genetics of HTN susceptibility and treatment[11,18,30,31], no scientifically-validated and commercially available panel exists to use genetics to guide therapy in these patients.

There is a well-described but clinically underappreciated bell-curve response to BP therapy. Each of the common classes of HTN therapy (diuretic, ACE-inhibitor, angiotensin-II receptor blocker, and Ca^{+} channel blocker) has a response rate of 40-50%, with placebo demonstrating a 25% effectiveness rate[4,10,12]. This medication effectiveness rate in HTN therapy goes beyond medication adherence, as the investigators in most previous studies reported adherence rates of 95%. Additionally, dose does not appear to dramatically affect response rates

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to common HTN therapy[23]. Importantly, while most patients demonstrate a drop in BP with HTN therapy, some patients demonstrate no change, and approximately 10-20% of patients demonstrate an increase in BP with drug treatment[4,12]. In particular, use of a diuretic and β-blocker have both demonstrated this clear bell-curve response, with an increase of BP in ~20% of patients, whether hydrochlorothiazide, metoprolol, or atenolol was used as a mono therapy, or as an add-on therapy[12]. Even though a portion of patients (~50%) do not respond to a given HTN therapy[11,32], and some may even experience an increase in BP, the current standard of care is to increase dosage of a given pharmacologic therapy to maximally tolerated dose and then layer a second HTN therapy upon the first drug which was not successful[14]. There are two very clear concerns with layering BP therapies when the first therapy does not work. First, there is a well-understood side-effect profile for each of the HTN therapies. For instance, long-term diuretic therapy leads to a 45% increase in the risk of development of new-onset diabetes when compared to ACE-inhibition[33,34]. In addition, previous work has demonstrated that β-blocker therapy results in an increase in insulin resistance and dyslipidemia[34]. Beyond increased risk of side effects, it has also been demonstrated that with each new HTN drug added as a therapy, there is a 70% reduction in medication adherence[35]. For clinicians, there may be some concern with removal of a drug that does not work, rather than layering on a new drug that may be successful. Interestingly, large studies that have involved a wash-out of an HTN therapy for research purposes find that more subjects in these studies (approximately 22-58% of patients) have to be removed from the study because of a drop in BP, rather than from an increase (9-27% of patients)[12,17].

Like most diseases, there is a genetic component to the development of HTN. The most compelling data currently suggests that HTN is ~50% heritable (with a range of 20-65%)[16].

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Several elegant studies have led to the estimate of the heritability of HTN by assessing the incidence rate in families, specifically twin studies and sons of HTN patients. The risk of developing HTN approximately doubles for each first degree relative who presents with HTN[19]. Schwartz et al. explored BP levels in sons of normotensive individuals when compared to sons of HTN patients[28]. Specifically, the work by Schwartz et al. demonstrated that sons of HTN patients were more likely to be HTN than sons of normotensive individuals, and that sons of HTN individuals had approximately 10 mmHg higher systolic and diastolic BPs when compared to sons of normotensive individuals. What makes the study by Schwartz et al. particularly compelling, is that 24-hr. holter data was used to assess ambulatory BP (rather than office or at home BP measures), and the investigators exposed the subjects to both a low (10 mEq/day) and a high (200 mEq/day) salt diet (each for two weeks), which was confirmed with urine Na^+ analysis[28]. Under each of the Na^+ intake conditions, the sons of HTN individuals demonstrated higher BP levels when compared to sons of normotensive individuals, and, interestingly, diet had very little statistical effect on BP. Additionally, previous work has demonstrated the strongest relationship to the prevalence of HTN in monozygous twins (~55%) when compared to dizygous twins (~40%), and both sets of twins were more likely to share the prevalence of HTN when compared to non-twin sibling pairs (~20-30%)[24,33]. Although the development of HTN clearly has a strong heritable component, like most disease states[18-20], environment also plays a role. When education level is taken into account, the relationship between the incidences of HTN in twins is significantly altered. Specifically, individuals with less than 14 years of formal education have a 17% reduction in the relationship between HTN incidence with their twin sibling (resulting in a 46% correlation coefficient), when compared to twin pairs with >14 years of education (who have a correlation coefficient of 63%)[36]. Although

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the heritability of the development of HTN is relatively clear, the genetic consequence of treatment is less clear.

Previous studies examining the genetic determinants to the response to HTN monotherapy and adjunctive therapies have primarily centered on the genetic variation of thiazide and thiazide-like diuretic response and include WNK1, alpha adducin, SLC12A3, and SCNN1A (which are also included in the present study)[12,18,25]. Specifically, genetic variation of WNK1 has demonstrated an approximately 5-6 mmHg difference in BP response to hydrochlorothiazide (re2107614, rs1159744, and rs2277869)[35]. The T variant of alpha adducin (rs4961) and C variant of SLC12A3 (rs1529927) have also been shown to be more responsive to a diuretic[37]. Similarly, the response to β-blockade has been studied in some detail. Previous studies in the heart and vasculature have demonstrated that the Gly16 (rs1042713) and Glu27 (rs1042714) variants of the ADRB2 and the AA49 (rs1801252) and CC389 (rs1801253) variants of the ADRB1 have enhanced receptor function in the cardiovascular system[38-41]. Because of this, pharmacogenetics specific studies have demonstrated that the GLy16/Glu27/Arg49/CC389 variants have an enhanced response to β-blockade, particularly when the genes are considered in combination[42,43]. Finally, the response to vasodilation has primarily focused on genetic variation of the ACE-inhibitor, angiotensin, and the angiotensin-II receptor. Specifically, the deletion variant of ACE (rs7079), the C variant of Angiotensin (rs699), and the C variant of the angiotensin-II receptor (rs5186) have shown enhanced response to ACE-inhibition and angiotensin receptor antagonism[44-46]. Collectively, these data demonstrate genetic variation may be partially responsible for the variability in effectiveness to HTN therapy and, possibly, the bell-curve response noted previously.

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One current area of focus for precision medicine is the pharmacogenetics of drug metabolizing enzymes (DME). Previous research has demonstrated that the assessment of genetic variation of DMEs alone does not significantly alter the BP response to β -blockade (one of the only common anti-hypertensive therapies to go through the cytochrome P450 pathway). Specifically, several previous studies have demonstrated that genetic variation of DMEs can significantly influence the amount of β-blockers in the plasma, but this does not significantly influence the BP response to these therapies[47-49]. Plasma levels in individuals who are slow metabolizers of β-blocker drugs has been shown to be as high as 20-fold greater than those who are fast metabolizers, but this difference did not influence the BP response to β-blockade[47]. These data further underscore the critical importance of receptor functionality and gene polymorphism determination of these receptors. Despite this, one promising area in the previous research is the coupling of the β_1 and β_2-adrenergic receptor functionality (the functional target receptor for selective and non-selective β-blockers, respectively) polymorphisms with CYP2D6 genotypes that are important in β-blocker metabolism. When coupled, previous research has demonstrated that Gly16 and Glu27 variants of the ADRB2 and Arg49 and Arg389 variants have a dramatic effect on BP response to β-blockade, particularly when coupled with CYP2D6*4 genotype status[50]. Data within the present study seems to confirm this (figure 1). We found no association between the CYP2D6 genotype and BP response to β-blockade in the present trial, when considered alone. However, there was an approximately 6 mmHg difference in SBP, DBP, and MAP between those patients who had a functional genotype of one of the adrenergic receptors and who were also on a β-blocker. This difference in BP is clinically meaningful and has previously been associated with a 40-50% reduction in myocardial infarction and stroke[51,52]. These data clearly demonstrate that coupling the drug target with the drug

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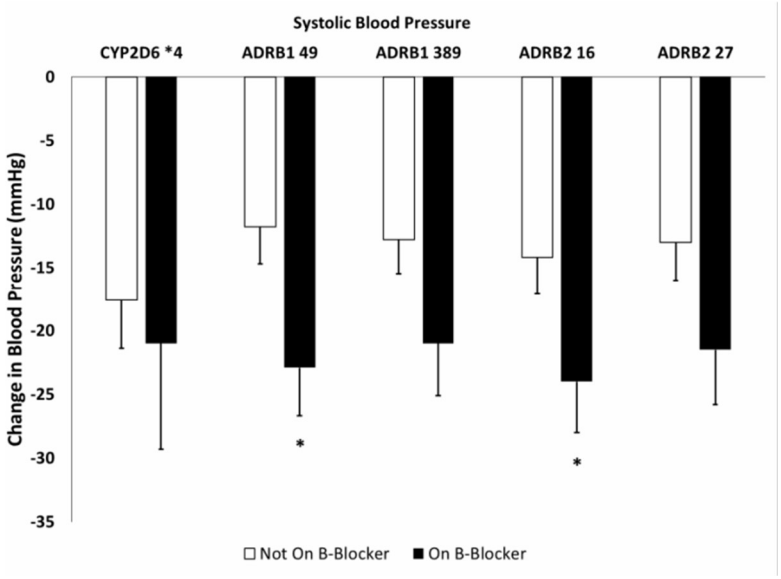
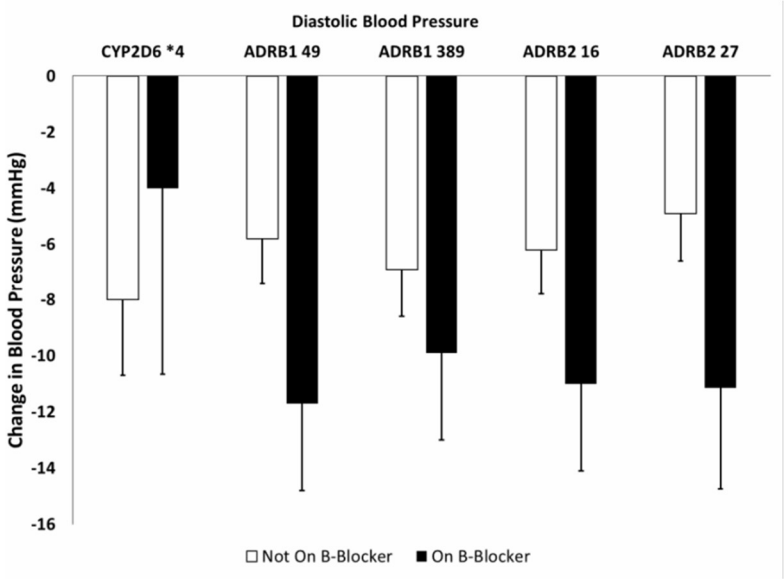
metabolizing enzyme may have a dramatic effect on BP reduction which is likely associated with improved survival.

Figure 1. Change in Blood Pressure for Functional Genotypes of Cytochrome P450 2D6 and the β1 and β_2-Adrenergic Receptors for Patients on a β-blocker vs. Patients not on a β-blocker.





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Limitations:

It is important to recognize potential limitations of this retrospective study. Despite the fact that we found important effects of the functional genotypes studied within the renal, vascular, and cardiac systems, and their target therapies, the sample size remains small due to the pilot nature of this work.,. Further, because of the relatively small sample size, the response BP as a function of gene polymorphism was variable and only statistically significant in about half of the genes examined, despite significant known functionality of these genes from previous much larger studies. In the present study, we only assessed the *4 variant because this has been specifically shown to influence the response to β-blocker and is common[47-49]. Other variants of CYP2D6 may also be significant, even if they occur in a smaller percentage of the population. Additionally, in the present study we assessed a gene-by-gene approach to determine the relationship between successful BP treatment and the genes of interest. This approach results in a high number of statistical comparisons and a virtual reduction in alpha due to correcting for these multiple comparisons. Additional studies need to be performed to expand the gene-by-gene approach to one that considers multiple genes simultaneously (i.e. functionality in angiotensin+ACE+A-II receptor should yield favorable A-II receptor response). In the present study, each patient was on an average of ~2 hypertension therapies. Given these limitations, future prospective randomized studies of larger scale (and use drug recommendations based on the gene panel as a whole, to preserve alpha) are important and should be undertaken.

Conclusion:

Our data, while preliminary in nature, demonstrate the effect of using multiple genes across multiple organ systems to direct therapy in patients with HTN. While larger studies are

 doi:10.20944/preprints201810.0028.v1

certainly needed, it is clear that this may help to alleviate some of the variability that exists in BP treatment and the relatively low success rates in this patient population.

Supportive/Supplementary Material

Table S1 describes the multiplex PCR primer pools, reaction, and cycling condition for step one of a two-step quantification of all gene polymorphisms with the exception of the ACE indel genotype. Table S2 describes the single base extension reaction primer pool, reaction, and cycling conditions for step two of the two-step quantification of gene polymorphisms. Table S3 describes the PCR primer, reaction, and cycling conditions for quantification of the ACE indel gene polymorphism.

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This study was supported by funds from Geneticure, Inc. which has developed the multi-gene panel for BP prescribing using pharmacogenetics. Eric Snyder, Ryan Sprissler, and Thomas Olson are co-founders of Geneticure. The study was completed in a blinded manner by a third-party research organization (RCRI, Inc.).

Author Contributions:

Conceptualization, Eric M. Snyder, Thomas P. Olson, and Ryan Sprissler; Data Entry, Greg D. Beenken and Micah Johnson; Data analysis, Eric M. Snyder, Thomas P. Olson, and Ryan Sprissler; Manuscript drafting and submission, Timothy Curry, Nicholas Cassuto, and Eli F. Kelley. No member from Geneticure was involved in data collection. The study was completed in a blinded manner by a third-party research organization (RCRI, Inc.).

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Conflict of Interest:

The study was supported by funds from Geneticure Inc. which has developed multi-gene panels for blood pressure prescribing using pharmacogenetics. Eric M. Snyder, Ryan Sprissler, and Thomas P. Olson have significant financial interest in Geneticure Inc. Micah Johnson, Greg D. Beenken, Timothy Curry, Nicholas Cassuto, and Eli F. Kelley declare they have no conflict of interest.

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Figure Legends:

Figure 1. Mean change in blood pressure for the cardiac functional genotypes (CYP2D6, ADRB1 49, ADRB1 389, ADRB2 16 and ADRB2 27) who are on a β-blocker (filled bars) vs. not (open bars). Sample sizes are provided in Table 5. *$p<0.05$ when compared to patients who are not on a beta-blocker.