Pharmacogenetics of Resistant Hypertension: Leveraging the Electronic Medical Record

By

Megan M. Shuey

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Approved:

Dan M. Roden, M.D.

Nancy J. Brown, M.D.

Alan R. Brash, Ph.D.

Joshua C. Denny, M.D.

James M. Luther, M.D., M.S.C.I.

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LIST OF ABBREVIATIONS

AA	African Americans
ACC/AHA	American College of Cardiology/American Heart Association
ACE	Angiotensin-converting enzyme
ACEi	Angiotensin-converting enzyme inhibitor
AF	Atrial fibrillation
AFl	Atrial flutter
ALLHAT	Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial
ARB	Angiotensin II receptor blocker
ARR	Aldosterone-renin-ratio
ASCOT-BPLA	Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm
BB	Beta blockers
BMI	Body mass index
BP	Blood pressure
CCB	Calcium channel blocker
CHF	Congestive heart failure
CKD	Chronic kidney disease
CKD3	Chronic kidney disease stage three
CKD-EPI	Chronic kidney disease epidemiology collaboration
CPT	Current procedural terminology
DBP	Diastolic blood pressure
DCT	Distal convoluted tubule
DHP	Dihydropyridine

DNA	Deoxyribonucleic Acid
EA	European Americans
EHR	Electronic health records
EMR	Electronic medical records
eGFR	Estimated glomerular filtration rate
eMERGE	electronic Medical Records and GEnomics
EMR	Electronic medical records
ENAC	Epithelial Na^+ channel
EPOGH	European Project on Genes in Hypertension
ExAC	Exome Aggregation Consortium
FDR	False discovery rate
GWAS	Genome wide association study
HbA1c	Hemoglobin A1c
HCTZ	Hydrochlorothiazide
HDL	High-density lipoprotein
HETE	Hydroxyeicosatetraenoic acid
HIPAA	Health Insurance Portability and Accountability Act
HRpEF	Congestive heart failure with preserved ejection fraction
ICD	International Classification of Disease
IHD	Ischemic heart disease
JHS	Jackson Heart Study
JNC 8	Eighth Joint National Committee
LDL	Low-density lipoprotein

LVEF	Left ventricular ejection fraction
MAF	Minor allele frequency
MDRD	Modification of Diet in Renal Disease
Misc antihtn	Miscellaneous antihypertensive
MR	Mineralocorticoid receptor
MVP	Million Veterans Program
NCC	Na^+-Cl^- cotransporter
NEC	Not elsewhere classified
NEDD4-2	Neural precursor cell-expressed developmentally downregulated (gene 4) protein
NKF-KDOQI	National Kidney Foundations-Kidney Disease Outcomes Quality Initiative
NOS	Not otherwise classified
NLP	Natural language processing
NPV	Negative predictive value
NSD	Novel start date
PAMELA	Pressioni Arteriose Monitorate E Loro Associazioni
PheKB	Phenotype KnowledgeBase
PCC	Pearson correlation coefficient
PLA_2	Phospholipase A_2
PL	Phospholipids
PPV	Positive predictive value
NADPH	Nicotinamide adenine dinucleotide phosphate
NCC	Sodium chloride (Na^+-Cl^-) cotransporter
NEC	Not elsewhere classified

NOS	Not otherwise classified
NPV	Negative predictive value
NSD	Novel start date
PATHWAY-2	Prevention And Treatment of Hypertension With Algorithm based therapY-2
PheWAS	Phenome-wide association study
PheKB	Phenotype KnowledgeBase
PL	Phospholipids
PLA_2	Phospholipase A_2
PPV	Positive predictive value
RAAS	Renin angiotensin aldosterone system
RAS	Renin angiotensin system
RH	Resistant hypertension
SBP	Systolic blood pressure
SD	Synthetic derivative
Sgk1	Serum- and Glucocorticoid-induced Kinase 1
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
TALH	Thick ascending limb of the loop of Henle
TIA	Transient ischemic attack
VA	Department of Veterans Affairs
VUMC	Vanderbilt University Medical Center

CHAPTER 1

INTRODUCTION

1.1 Resistant Hypertension

Hypertension, or elevated blood pressure (BP), is a pervasive global health problem and contributes to 13.5% of deaths worldwide.[1] In 2010, the worldwide prevalence of hypertension among persons over the age of 20 years was estimated to be 31.1%.[2] In randomized controlled clinical trials, treatment with antihypertensive medications reduces many of the risks associated with hypertension.[3] Effective BP treatment often requires concurrent use of several antihypertensive drugs affecting complementary pathways.

While control of BP reduces morbidity and mortality in patients with hypertension, in a subset of patients the usual antihypertensive medication intervention does not adequately control BP. Among patients with hypertension, it is estimated that 69% have uncontrolled BP despite antihypertensive use, according to the National Health and Nutrition Examination Survey 1999 to 2000.[4] Some of these patients with uncontrolled BP have resistant hypertension (RH), defined as persistently elevated BP despite concurrent use of three antihypertensive medications, including a thiazide diuretic.[5]

The exact prevalence of RH among hypertensive patients is not known; however, clinical trials and epidemiologic studies have suggested the prevalence to range from 8.4% to 50%.[6-13] Compared to patients with controlled hypertension, patients with RH are at an elevated risk of chronic kidney disease (CKD), stroke, and cardiovascular events.[5, 14, 15] In some RH patients, inadequate treatment and medication nonadherence contribute to inadequate BP control; these patients have pseudo-resistant hypertension.[16] Causes of inadequate treatment include the use of

less than optimal doses of antihypertensive medications[17] or the underutilization of preferred antihypertensive therapies such as chlorthalidone or spironolactone.[18] For patients with true RH, however, persistently increased BP may result from underlying molecular mechanisms that are not targeted by or attenuate the response to usual antihypertensive therapies. We hypothesized that one such mechanism may involve the cytochrome P450 enzyme CYP4A11, a hypothesis that we develop in Section 1.4.

1.2 Racial Differences in Hypertension

Race plays an important role in the development and outcomes of hypertension. African Americans (AA) exhibit higher rates of hypertension than European Americans (EA) and hypertension is often more severe.[19-23] Hypertension also develops at an earlier age in AA than in EA, and in a study of US children, aged 8-17 years, AA boys and girls had significantly higher systolic blood pressure (SBP) than EA boys and girls.[24-26] In addition to higher rates of hypertension, the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) demonstrated that AA participants had more treatment resistance, defined as uncontrolled BP on an average of two medications, than EA.[27] Further, in a community-based study of AA from the Jackson Heart Study (JHS), masked hypertension, defined as normal clinical BP but elevated home BP collected by 24-hour ambulatory BP monitoring, occurred in one-third of the patients presenting with controlled clinic BPs.[28] There is a higher prevalence of masked hypertension in the JHS than in other population-based studies from Japan, Denmark, seven countries from the European Project on Genes in Hypertension (EPOGH), and the Pressioni Arteriose Monitorate E Loro Associazioni (PAMELA) study in Italy.[29-32]

Disproportionate levels of hypertension in AA may explain some of the racial disparities in mortality and hypertension-related disease risk.

In general, AA are at an increased risk of mortality and studies of racial disparities in mortality rates suggest that much of the discrepancy can be explained by hypertension.[33] In a follow-up study of EA and AA hypertensive patients, the population attributable risk of 30-year risk of mortality due to hypertension was 23.8% and 18.3% in EA men and women compared to 45.2% and 39.5% in AAs, respectively.[34] Further, AA hypertensives are at an increased risk of developing stroke, congestive heart failure, and CKD compared to EA hypertensives.[35-41]

While AA are at an increased risk for the development of many hypertension related diseases there are some conditions that occur at lower rates in AA than EA. For example, AA exhibit substantially lower rates of atrial fibrillation (AF) and atrial flutter (AFl) than EA and hypertension is a risk factor for AF/AFl.[42-44] This paradox remains unexplained. In the ALLHAT trial there was a significant association between pre-existing and new-onset AF/AFl and increased mortality in a population of high-risk hypertensives.[44]

These disparities in cardiovascular and renal risks may impact the prescription of various antihypertensive medications. In **Chapter 2** we will discuss the prevalence of these conditions in patients with resistant hypertension as well as how they may impact prescription use.

1.3 Antihypertensive Therapies for Blood Pressure Control

The pharmacologic treatment of hypertension often requires that patients simultaneously use several antihypertensive medications that target different molecular pathways to achieve adequate BP control (**Figure 1**). Randomized, controlled trials support the use of different antihypertensive drug treatment strategies to control BP and improve patient outcomes. This

evidence is then used in the development of clinical guidelines for the treatment of hypertension, such as those presented by the Panel Members Appointed to the Eighth Joint National Committee (JNC 8).[45] Under the 2014 guidelines proposed by JNC 8 initial treatment strategies for nonblack hypertensive patients should include a thiazide-type diuretic, calcium channel blocker (CCB), angiotensin-converting enzyme inhibitor (ACEi), or angiotensin receptor blocker (ARB). In hypertensive AA, however, initial treatment should include a thiazide-type diuretic or CCB. These guidelines also provide suggestions for specific subgroups of antihypertensive patients including those with CKD. The most recent guidelines presented by the American College of Cardiology/American Heart Association (ACC/AHA) Task force in fall of 2017 provide a more up to date guideline for medication treatment and hypertension assessment. Consistent with previous guidelines, unless other indications are present, the first line antihypertensive medication treatment should include an ARB or ACEi, CCB, or thiazide diuretic. In AA, CCBs and thiazides are the preferred first-line agents, whereas beta-blockers and renin-angiotensin-aldosterone system (RAAS) blockers, including ACEis and ARBs, are not preferred.[46]

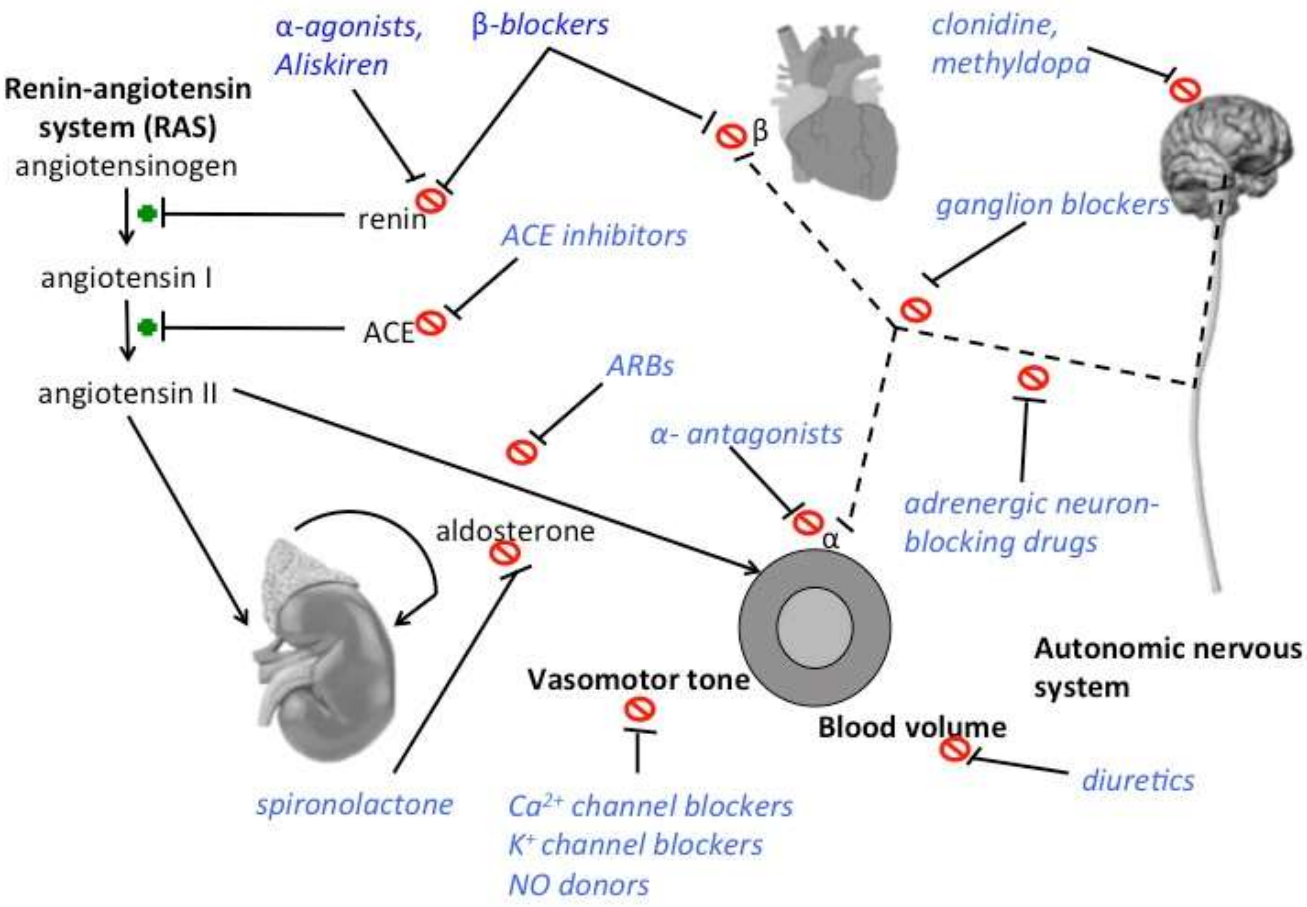

Figure 1. Antihypertensive medication classes and pathways for blood pressure control. The different pathways that can affect blood pressure (BP) are bolded while the different antihypertensive medication classes appear in blue italics. Some classes of antihypertensive medications lower BP by inhibiting the renin-angiotensin-aldosterone system (RAAS). The first step of the RAAS, conversion of angiotensinogen to angiotensin I, can be blocked by the inhibition of renin. Renin can be inhibited either directly by aliskiren or by modulating renin secretion by activating the inhibitory α-2 receptor with α-2 agonists or by blocking the β_1-adrenoceptors with β blockers. Angiotensin-converting enzyme (ACE) inhibitors block the next step of the RAAS pathway, conversion of angiotensin I to angiotensin II. Angiotensin II receptor blockers (ARBs) block the activation of AT_1 receptors by angiotensin II, which in turn results in

vasodilation and reduces the production and secretion of aldosterone. Mineralocorticoid receptor (MR) antagonists, such as spironolactone prevent aldosterone from activating the MR. Vasomotor tone determines the pressure within blood vessels and Ca^{2+} channel blockers (CCBs), K^{+} blockers, and nitric oxide donors decrease systemic vascular resistance, which lowers arterial BP. Diuretics decrease blood volume by inhibiting renal channels resulting in natriuresis and a subsequent decrease in BP. The observation that most Mendelian forms of hypertension involve genetic variants that affect renal sodium handling speaks to the importance of this pathway. Finally, modulation of the autonomic nervous system by clonidine and other α2- agonists lower peripheral vascular resistance, which in turn lowers BP.

In patients with RH, the simultaneous use of several antihypertensive medications at maximum tolerable doses defines patients with true RH.[47] For patients with RH, international guidelines have converged on a treatment strategy requiring simultaneous use of the maximum tolerated doses of an ACEi or ARB, a CCB, and a thiazide diuretic.[5, 48-50] Further, additional clinical trials have been used to identify preferred add-on therapies to the aforementioned regimes including the use of the aldosterone antagonist spironolactone.[51-53] Unfortunately, medication non-adherence in patients with RH can confound true resistance.[54-58] The use of assays to detect medications and metabolites in a patient's plasma or urine can confirm medication adherence and differentiate true from apparent RH.[56, 59] In patients with true RH, the identification of potential novel pharmacologic targets for BP control is of particular interest.

1.4 The Cytochrome P450 Hydroxylase Pathway and Blood Pressure Regulation

Members of the cytochrome P450 family of enzymes, CYP4A and CYP4F, catalyze the nicotinamide adenine dinucleotide phosphate (NADPH)-dependent ω-hydroxylation of arachidonic acid to form 19- and 20-hydroxyeicosatetraenoic acid (HETE),[60] illustrated in **Figure 2**. In humans CYP4A11 and CYP4F2 are responsible for the formation of 20-HETE. In the kidney 20-HETE can exert BP-raising and -lowering effects.

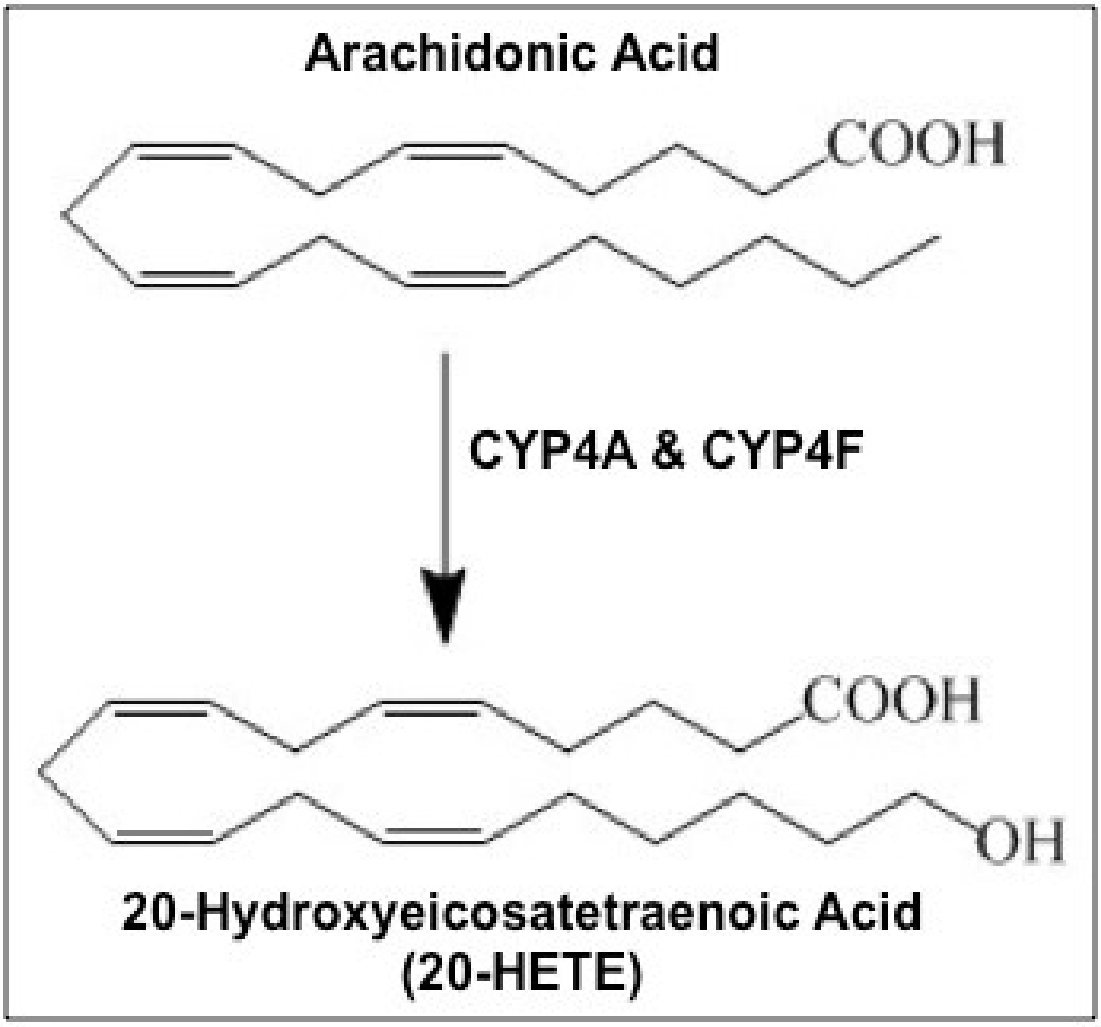

Figure 2. Metabolism of Arachidonic Acid to 20-hydroxyeicosatetraenoic acid CYP4A and CYPF enzymes metabolize arachidonic acid to 20-hydroxyeicosatetraenoic acid.

In vascular smooth muscle cells 20-HETE causes vasoconstriction.[61-63] Vasoconstriction of the renal microtubules or peripheral vasculature promotes an increase in BP.[64] Specifically, 20-HETE depolarizes vascular smooth muscle cells by inhibiting the Na^+-K^+ ATPase and decreasing the open probability of Ca^{2+}-dependent K^+ channels.[65] Independent of this depolarization, 20-HETE can also activate L-type Ca^{2+} channels by a PKC-dependent mechanism.[66, 67] These actions of 20-HETE in vascular smooth muscle cells lead to vascular contraction, vasoconstriction, and an increase in BP (**Figure 3**). 20-HETE can also potentiate the effects of other vasoconstrictors by inactivating calcium-sensitive potassium channels in smooth muscle cells and increasing the expression of ACE.[68]

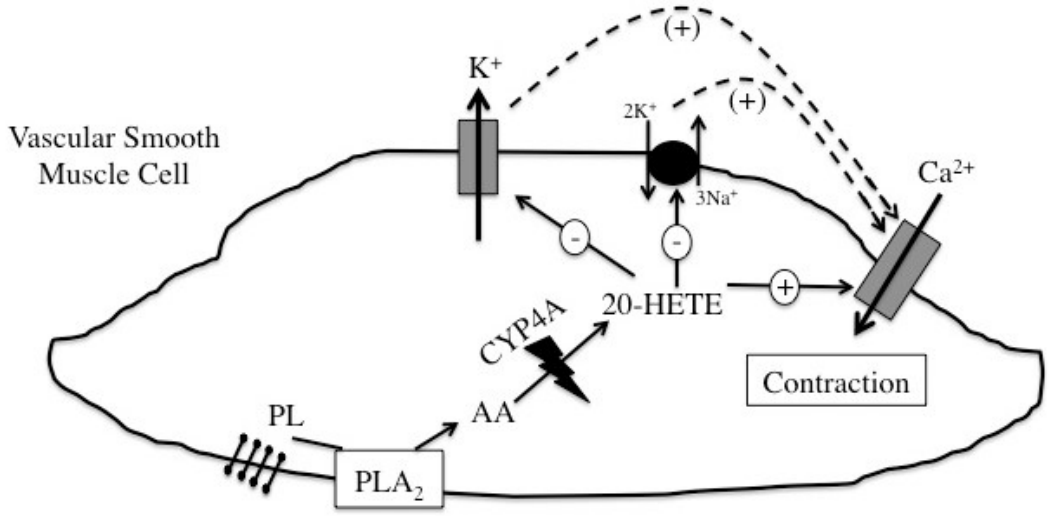

Figure 3. Function of 20-hydroxyeicosatetraenoic acid in vascular smooth muscle cells. In vascular smooth muscle cells the activation of phospholipase A_2 (PLA_2) results in the hydrolysis of the sn-2 acyl bond of membrane phospholipids (PL) and the release of arachidonic acid (AA). The arachidonic acid is then hydrolyzed by CYP4A to form 20-HETE. 20-HETE inhibits the Na^+-K^+ ATPase and calcium-dependent K^+ channels resulting in depolarization that in turn activates L-type Ca^{2+} channels. Further, 20-HETE can directly activate L-type Ca^{2+} channels. The activation of the L-type Ca^{2+} channels leads to an influx of Ca^{2+} that initiates contraction.

In contrast, effects of 20-HETE in the renal tubules can decrease BP by increasing Na^+ excretion or natriuresis. 20-HETE increases Na^+ excretion by inhibiting both the Na^+-K^+ ATPase in the proximal tubule and thick ascending limb of the loop of Henle (TALH)[69-72] and the Na^+-K^+-$2Cl^-$ co-transporter in the TALH,[73] **Figure 4**. Various classes of antihypertensive medications, including loop, K^+-sparing, and thiazide diuretics, as well as mineralocorticoid receptor antagonists, act through the same mechanism of increasing natriuresis by the inhibition of these channels as well as others to decrease BP (**Figure 4**).

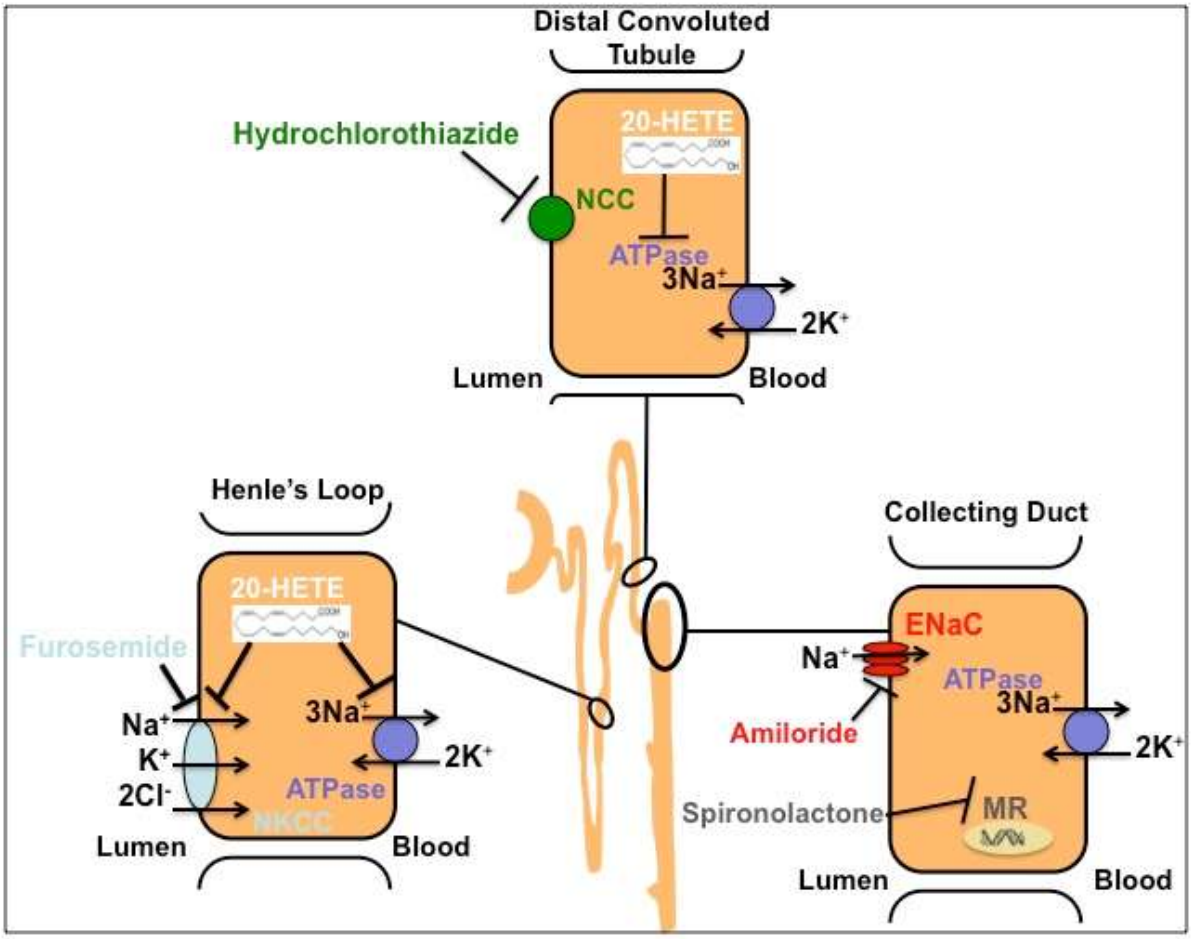

Figure 4. **Targets of 20-HETE and diuretic drugs in the renal tubule**. In the renal tubules, 20-HETE and various diuretics increase natriuresis. In the thick ascending limb of the loop of Henle (Henle's Loop), 20-HETE inhibits the Na^+-K^+-$2Cl^-$ co-transporter as well as the Na^+-K^+ ATPase. Loop diuretics also inhibit the Na^+-K^+-$2Cl^-$ co-transporter. In the distal convoluted tubule, 20-HETE inhibits the Na^+-K^+ ATPase and thiazide diuretics inhibit the Na^+-Cl^- cotransporter (NCC). In the collecting duct, antagonism of the mineralocorticoid receptor (MR) prevents activation of the epithelial Na^+ channel (ENaC) and other K^+-sparing diuretics such as amiloride and triamterene inhibit ENaC directly.

Antihypertensive medication classes designed to target the channels and cotransporters that regulate urine volume and sodium excretion in the kidney nephron are called diuretics. The human kidney is comprised of approximately a million nephrons, the primary urine forming unit of the kidney. Throughout the nephron are specific segments comprised of different channels and transporters that regulate urine formation. Loop diuretics, such as furosemide, work primarily by inhibiting the luminal Na^+-K^+-$2Cl^-$ co-transporter in the TALH. Inhibition of the Na^+-K^+-$2Cl^-$ co-transporter results in an almost complete decrease of salt transport in this portion of the nephron, which equates to a decrease of up to 25% of the total filtered load of Na^+.[74] In the distal convoluted tubule (DCT), thiazide and thiazide-like diuretics target the luminal Na^+-Cl^- cotransporter (NCC). Inhibition of the NCC results in a moderate increase in Na^+ excretion, approximately 5% of the total filter load.[74] Both loop and thiazide/thiazide-like diuretics result in an increase in K^+ excretion, which can cause hypokalemia if dietary K^+ intake is not substantially increased. In the final section of the nephron, the collecting duct, K^+-sparing diuretics work by directly inhibiting the epithelial Na^+ channel (ENaC) or by decreasing ENaC expression by antagonizing the mineralocorticoid receptor (MR). Inhibiting luminal ENaC or decreasing ENaC expression by K^+-sparing diuretics results in a mild increase in Na^+ excretion, but the resulting hyperpolarization of the membrane results in a decrease in the excretion K^+, in contrast to the effects of other diuretic classes.[74] The effects of 20-HETE in the nephron may mirror those of the loop diuretics resulting in a similar increase in natriuresis, decrease in blood pressure, and potential for hypokalemia.[75]

In humans, the antihypertensive effects of 20-HETE appear to predominate over the prohypertensive effects based on the observation that carriers of a loss-of-function variant, c.8590C>T (rs1126742), of the gene encoding the CYP4A11 enzyme, *CYP4A11*, exhibit salt-

sensitive hypertension.[76] The predominance of the antihypertensive effect is further supported by a lack of association of c.8590C>T genotype with vasoconstriction following systemic Ang II or norepinephrine infusion.[73, 76, 77] The c.8590C>T variant has been associated with a significant decrease in urinary 20-HETE excretion in humans in one study, supporting *in vivo* the loss-of-function phenotype in carriers of this *CYP4A11* variant.[78]

1.5 Genetic variants of *CYP4A11* are associated with hypertension and an attenuated response to spironolactone.

The *CYP4A11* gene comprises 12,326 base pairs located on chromosome 1 and encodes the CYP4A11 protein comprised of 519 amino acids. Throughout the entire gene region, including both coding and non-coding region, 1,389 single nucleotide polymorphism (SNP) variants of *CYP4A11* have been identified, including a non-synonymous variant located on exon 11, rs1126742 (c.8590C>T resulting in Phe434Ser), which results in a 50% decrease in 20-HETE synthase activity *in vitro*. [79] From the Genome Aggregation Database (gnomAD), the frequency of the minor C allele of rs1126742 is 31.6% and 13.9% in the African and European (non-Finnish) populations, respectively.[80] This variant has been associated with elevated BP or hypertension in the Framingham Offspring Trial and a Tennessee case-control study,[79] in males in the western Chinese Han population,[81] in AA men with hypertensive nephrosclerosis,[77] in European survivors of myocardial infarction,[82] and a European population survey.[83] The C allele of rs1126742 is also associated with salt-sensitive hypertension in humans.[76]

Other research has identified a variant in intron 10 of *CYP4A11*, rs3890011, which is in partial linkage disequilibrium with rs1126742.[84] From the gnomAD, the frequency of the C allele of rs3890011 is 58.8% and 21.8% in the African and European (non-Finnish) populations,

respectively.[80] This variant was nominally associated with systolic blood pressure (SBP) in normotensive Chinese men, and haplotypes containing the C allele were associated with hypertension in women.[85] In addition, the C allele of rs389011 was associated with an attenuated response to the MR antagonist, spironolactone, but not to the ENaC inhibitor, amiloride, in 83 hypertensive AA with low-renin hypertension.[86] An association between spironolactone response and the rs1126742 genotype was not evaluated because the low minor allele frequency (MAF) of rs1126742 precluded evaluation in the small study group.[86] These findings suggest that a product of the CYP4A11, such as 20-HETE, may affect ENaC activation, and may suggest the potential to predict individuals who should or should not be treated with a direct ENaC inhibitor as opposed to a MR antagonist.

Taken together, the association of *CYP4A11* loss-of-function variants with BP, hypertension, and an attenuated response to MR antagonists, the suggested fourth line treatment for true RH,[87, 88] suggests the hypothesis that the CYP4A11 pathway represents a novel pathway underlying RH that is not targeted by current pharmacological strategies.

1.6 The identification of a clinical RH patient population using electronic medical records

Genetic association studies in complex diseases such as RH require large sample sizes. Fortunately, the development of electronic medical records (EMRs)[89] provides the opportunity to access substantial quantities of clinically relevant data for research. One potential use of these data is for the classification of clinically relevant phenotypes, such as RH, for use in genetic research.

When using EMRs in research for phenotype creation it is important to note that EMRs were not designed explicitly for research. Because EMRs were designed for the documentation

of clinical observations, documentation of patient-clinician interactions, and billing there are substantial challenges with EMR use for research.[90-92] For example, collected data may be incomplete or inaccurate,[93] which may decrease the predictive power of a developed phenotype.[94] Complex data collection including a mix of continuous and discrete variables, coupled with multiple collection approaches that may require variable identification using natural language processing (NLP) techniques, can further complicate the creation of phenotypes. NLP is a field of computer science that deals with the manipulation of natural language, spoken or written word, by software. Essentially a computer system uses a set of rules/guidelines to extract information from notes written by a clinician. Human language is complex, however, so misspellings and abnormal sentence structure can result in inaccurate interpretations by software. Therefore, while EMRs have the potential to advance research, it is essential to understand the challenges and limitations of the resource for the creation of the clinical phenotypes.

Despite these challenges there are advantages to studies using the EMR. Because the EMR is derived from a real-world clinical population the findings in studies using EMRs may readily translate to other clinics. Further, the structure of the data provides researchers the ability to easily curate large datasets to address clinical questions. Finally, EMR use allows researchers to identify a large number of patients with specific clinical conditions, such as resistant hypertension, and address research questions for which sample size is typically prohibitive.

1.7 Vanderbilt University Medical Center's electronic medical records and DNA biorepository

At Vanderbilt University Medical Center (VUMC) a subset of the EMRs are linked to DNA samples. This DNA biobank, BioVU, can be used for the identification of cases and controls using EMR-derived phenotypes and subsequent genetic association studies. BioVU links de-identified patient information from EMRs to DNA extracted from leftover clinical blood samples (**Figure 5**).[95] As of September 2017, BioVU includes samples from 212,080 adults and 29,664 children. Further, there are genome-wide association study (GWAS) level data for 43,716 of these subjects. At present, the BioVU resource has been used to replicate or discover new genomic markers for many traits including electrocardiogram intervals, type 2 diabetes mellitus, AF, rheumatoid arthritis, Cohn's disease, multiple sclerosis, and hypothyroidism.[96-99] It has also enabled the development of new methods such as Phenome-Wide Association studies (PheWAS).[100]

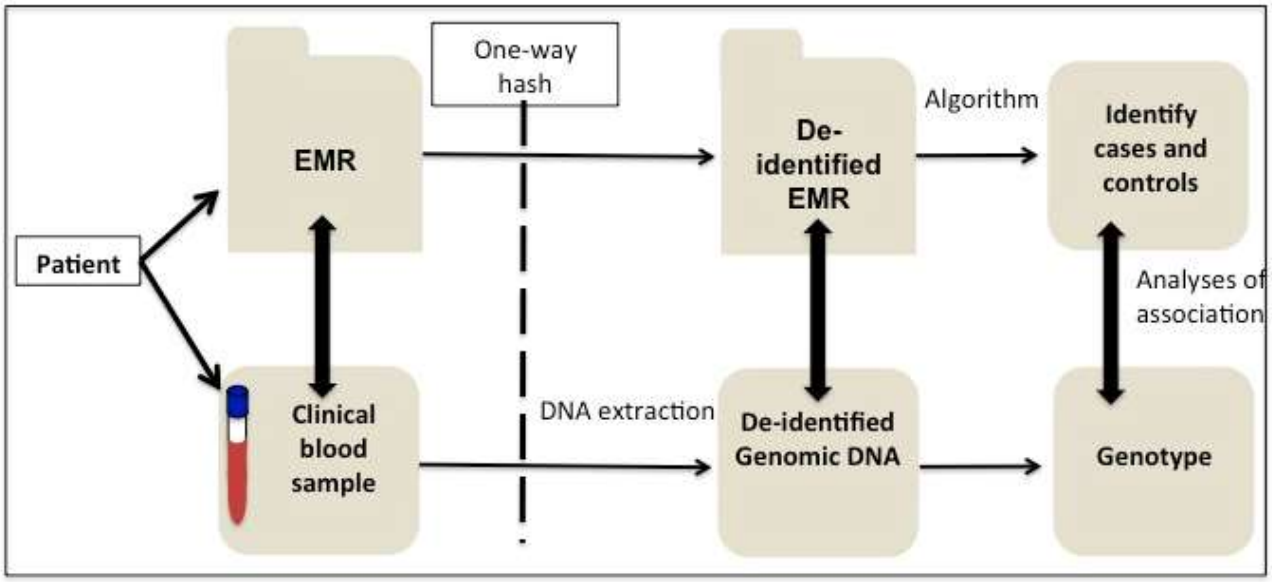

Figure 5. Diagram of Vanderbilt University Medical Center's electronic medical record linked DNA biobank, BioVU. When a patient checks in at a Vanderbilt University Medical Center (VUMC) outpatient clinic the subject may choose to participate in BioVU by signing a consent form. After a patient consents to participate in BioVU, his or her physician may at some point during normal care order routine blood tests in the outpatient setting. After clinical testing is performed on these blood samples, and if there is leftover blood, the sample is tested to determine BioVU eligibility. If the sample is BioVU eligible, then DNA is extracted from the leftover blood and samples are stored. The electronic medical record (EMR) and clinical blood sample each go through a one-way cryptographic hash protocol to create an identifier to link the two together, and then the EMR is scrubbed of fields specified in Section 164.514 of the HIPPA privacy rule, e.g. identifiers, using combinations of natural language processing and other methods. The EMR exists as a combination of structured data, e.g. ICD 9/10 diagnostic codes, laboratory tests, and unstructured data, e.g. procedural notes, that can be used to create algorithms to identify specific phenotypes or subsets of the clinical population. We will discuss this process in terms of the identification of patients with resistant hypertension in **Chapter 2**. The phenotype-identified subjects with linked DNA can then be used in genetic association studies to identify novel mechanisms underlying a specific condition. We will discuss this later process in **Chapter 3** using a different DNA biobank, the Department of Veteran Affairs Million Veterans Program (VA MVP).

Because BioVU was initiated in 2007 and includes only a subset of the patients in the larger EMR (many of whom have longitudinal data collected beginning in the early 1990's) there may be differences in patient populations derived solely from BioVU as opposed to the larger EMR. These differences may reflect more recent changes in clinical practice that are largely absent in the larger EMR, e.g. changes in prescribing practices due to a new therapeutic coming on the market or a recent clinical trial suggesting the use of a particular medication for a given condition. Therefore, these potential changes in clinical practice may result in significant differences in the patients identified by a particular phenotype in BioVU when compared to those identified in the larger EMR. We discuss some of these clinical differences in patients identified in the VUMC BioVU compared to the larger EMR in the context of the RH phenotype in greater detail in **Chapter 4**.

1.8 Dissertation overview

This dissertation develops methodology to test the hypothesis that ***CYP4A11* loss-of-function variants are associated with RH due to attenuated responses to specific diuretics, including spironolactone**. To address this hypothesis I developed and validated an EMR-derived phenotype algorithm for RH in the VUMC EMR for the identification of RH cases and easily-controlled hypertension controls (**Chapter 2**). A subset of these cases and controls are part of BioVU and will be used for subsequent studies including, 1) an estimation of the frequency of medication non-adherence among the RH population using a mass spectrometry assay; 2) a genetic association study of the *CYP4A11* loss-of-function variants and RH (**Chapter 3**); and 3) an genetic association study of the *CYP4A11* loss-of-function variants and spironolactone response.

The methods developed for this dissertation open doors for future studies. Specifically, the RH case-control population identified in **Chapter 2** of this dissertation provides a robust phenotype for use in future genetic and epidemiologic studies of RH. The identification of an association between the loss-of-function *CYP4A11* variants and clinical RH suggest that the 20-HETE metabolic pathway and effects of 20-HETE within the kidney nephrons are potential targets for novel RH therapeutics (**Chapter 4**). The algorithm for spironolactone response utilized in **Chapter 4** offers a novel approach to test for an association between a specific genotype and BP response to a specific medication using curated EMR data that may result in new strategies to personalize medication prescribing.

CHAPTER 2

CHARACTERISTICS AND TREATMENT OF AFRICAN AMERICAN AND EUROPEAN AMERICAN PATIENTS WITH RESISTANT HYPERTENSION AT VANDERBILT UNIVERSITY MEDICAL CENTER

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2.1 Abstract

The identification of hypertensive patient subpopulations using electronic health records (EHR) can enable genetic studies and elucidate practices in the real-world clinical treatment of hypertension. Using Vanderbilt University Medical Center's (VUMC's) EHR, we developed algorithms to identify patients with resistant hypertension, cases, and easily-controlled hypertension, controls, for use in future genetic studies. Among 186,015 European American (EA) and 33,576 African American (AA) hypertensive patients, 13,541 (7.3%) and 3,541 (10.5%) had resistant hypertension, respectively. AA with or without resistant hypertension were younger, heavier, more often female, and had a higher incidence of type 2 diabetes, and higher systolic and diastolic blood pressures than EA. AA with resistant hypertension were more likely than EA to be treated with vasodilators, dihydropyridine calcium channel blockers, and alpha-2 agonists. EA were more likely to be treated with angiotensin receptor blockers, renin inhibitors, and beta blockers. Mineralocorticoid receptor antagonists were used in both EA and AA patients and use was increased in patients treated with more than four antihypertensive medications compared to patients treated with three (12.4% vs 2.6% in EA, $p<0.001$; 12.3% vs

2.8% in AA, p<0.001). The number of patients treated with a mineralocorticoid receptor antagonist increased to 37.4% in EA and 41.2% in AA over a mean follow-up period of 7.4 and 8.7 years, respectively. These results demonstrate the feasibility of identifying resistant hypertension using an EHR and that treatment of resistant hypertension differs in EA and AA patients at an academic center.

2.2 Introduction

Patients with resistant hypertension, defined as persistently elevated blood pressure despite concurrent treatment with three or more different antihypertensive medications including a thiazide diuretic, are at an elevated risk of chronic kidney disease (CKD) and a 47% higher risk of cardiovascular events compared to patients with controlled hypertension.[5, 14] The estimated prevalence of resistant hypertension ranges from 8.4% to 50% of all treated hypertensive patients in clinical trials and epidemiologic studies due to varying definitions of resistant hypertension and methods of blood pressure (BP) assessment.[6-13, 101] A meta-analysis by Achelrod et al. estimated a prevalence of resistant hypertension of 13.72% in 20 observational studies and 16.32% in four randomized control trials.[102] These rates are consistent with a separate meta-analysis of North American and European studies that reported a resistant hypertensive prevalence rate of 14.8% in treated hypertensive patient.[12] These rates may be inflated because studies did not assess adequacy of drug treatment or medication non-adherence.

The rates of inadequate drug treatment or medication non-adherence are not known precisely for the population. In a study by Egan et al. half of the patients with uncontrolled BP on greater than three antihypertensive medications were prescribed optimal treatment and patients with greater cardiovascular risk were more often prescribed optimal treatment.[47]

Medication nonadherance, in particular, may lead to overestimation of the prevalence of resistant hypertension. Studies assessing medication adherence using blood and urine levels to measure antihypertensive medication intake estimate the rate of non-adherence, including partial and complete non-adherence, to be approximately 50% in resistant hypertensive patients and 25% in hypertensive patients with uncontrolled BP.[56, 59, 103] These conditions may contribute to uncontrolled blood pressure in some but not all patients with resistant hypertension. The molecular mechanisms underlying resistant hypertension in the remaining population remain unknown. Therefore, understanding the genetic underpinning of resistant hypertension versus readily controlled hypertension is of great value.

To date, however, the identification of novel genetic underpinnings of population-wide hypertension has met with limited success. Genome-wide association studies (GWAS) can analyze up to millions of genetic variants from across the human genome in an effort to identify genetic risk factors of disease. GWAS studies of BP and hypertension have identified a number of variants that associate with BP and hypertension.[104-108] The contribution of these variants to the overall heritability of hypertension, however, has been relatively small.[109-111] Conversely, studies of Mendelian forms of hypertension have identified novel rare variants with large effect size that contribute to hypertension.[109, 112-115] These findings have provided mechanistic insight into the etiology of hypertension. Resequencing efforts in the Framingham Heart Study have further supported the role for some of these rare variants in BP regulation.[116] Because much is still left to learn about the genetic contributions to hypertension there is a need to study more severe hypertension in large datasets for which clinical and medication data are available. Electronic health record (EHR) linked with genetic material provides a robust resource to do just that.[117, 118]

We hypothesized that we could use the EHR to develop algorithms to identify patients within a clinical population who had resistant hypertension or easily controlled hypertension for use in future genetic studies. Resistant hypertensive cases were defined as patients with BP $\geq$ 140/90 mmHg despite concurrent use of three or more antihypertensive medications including a thiazide, thiazide-like diuretic, or a dihydropyridine calcium channel blocker (DHP CCB) or those taking four or more antihypertensive medications including a thiazide, thiazide-like diuretic, or DHP CCB. Controls with controlled hypertension were defined as patients with blood pressure $\leq$ 135/90 mmHg on one and only ever one medication. Our case and control definitions were designed to be stringent; excluding secondary causes of hypertension and CKD stages four and five, to select a more homogenous population for use in genetic studies. The accuracy of the algorithms was validated by chart review. In developing the method we observed differences in the patterns of prescribing of antihypertensive medications between AA and EA patients with resistant hypertension. We describe here the characteristics and medication treatment of AA versus EA patients with resistant hypertension at VUMC.

2.3 Methods

Electronic health record

We used the Vanderbilt University Medical Center (VUMC) Synthetic Derivative (SD) for this research. The SD is a de-identified copy of the VUMC EHR system with Health Insurance Portability and Accountability Act (HIPAA) identifiers removed by established de-identification software as well as custom algorithms.[119] The Institutional Review Board reviewed this project and deemed it exempt as non-human subjects research in accordance with 45CFR46. To date the SD contains approximately 2.5 million records and approximately one million of

these records contain detailed longitudinal data. The SD contains almost all available clinical data including basic demographics, such as race and sex; text from clinical care notes; laboratory values; inpatient and outpatient medication data; international classification of disease (ICD) and current procedural terminology (CPT) codes; and other diagnostic reports.

Resistant hypertension algorithm development

To identify patients with resistant hypertension (cases) and patients with controlled hypertension (controls) in the VUMC SD, we developed the following algorithms, updated and modified from a previously published algorithm to define resistant hypertension within the electronic MEdical Records & GEnomics (eMERGE) network.[105, 120]

Resistant hypertension was defined by one of two possible case definitions. Case Type I identified patients with elevated blood pressure despite simultaneous treatment with at least three different classes of anti-hypertensive medications, including a thiazide diuretic, amlodipine or other DHP CCB (**Figure 6).** Because thiazide-induced hyponatremia is a relatively common clinical condition and clinical guidelines suggest thiazide or CCB use, we allowed for the replacement of thiazide diuretics with DHP CCBs.[121-124] Antihypertensive medication classes were angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), beta blockers (BBs), alpha-2 agonists, CCBs, thiazide and thiazide-like diuretics, aldosterone antagonists, other non-thiazide diuretics, direct-acting vasodilators, alpha antagonists, renin inhibitors, and miscellaneous antihypertensives (**Table 1**)**.** In the case of combination therapies, each component antihypertensive medication was counted separately. Simultaneous treatment with three different classes of antihypertensive medication was confirmed by documentation in the EHR on two occasions separated by more than one month.

Uncontrolled hypertension was defined by at least two recorded outpatient measurements of systolic blood pressure (SBP) greater than 140 mmHg or diastolic blood pressure (DBP) greater than 90 mmHg at least one month after medication criteria were met, as well as by a mean outpatient SBP or DBP calculated as greater than 140/90 mmHg during the six months after the medication criteria were met.

The Case Type II definition of resistant hypertension identified patients who were treated simultaneously with four or more classes of antihypertensive medication, including a thiazide-type diuretic, amlodipine, or other DHP CCB, as documented on at least two occasions more than one month apart, regardless of blood pressure. Patients who met the criteria for Case Type I but subsequently met the definition of Case Type II by virtue of having a new medication class added were considered to meet the definition of Case Type II resistant hypertension for all analyses.

Controls, patients with controlled hypertension, were defined as patients who had been assigned an International Classification of Disease (ICD)-9 or -10 code for hypertension (401.* and I10, respectively) and who were treated with a single antihypertensive medication. The control definition required that at least one blood pressure be recorded in the month following the initiation of the antihypertensive medication and that all recorded SBP and DBP in the month were less than 135 and 90 mmHg, respectively. In addition, these patients never received concurrent treatment with more than one antihypertensive class at any time in the EHR. Therefore, control patients could never be classified as a case at a different time in the EHR.

For all three algorithms, drug exposure to antihypertensive medications was identified in the VUMC SD by electronic-prescribing tools and by using MedEx,[125] a natural language processing tool that recognizes medication names and information including drug dose, route,

frequency, and duration from unstructured clinical documents. I required the presence of at least one of the following identifiers-drug dose, route, frequency, or duration- for a MedEx-identified drug to be counted. The utility of these tools for extracting medication data from the EHR has been shown previously.[126, 127]

All patient characteristics- age, gender, body mass index (BMI), blood pressures, and history of type 2 diabetes mellitus (T2DM), sleep apnea, ischemic heart disease (IHD), transient ischemic attack (TIA), congestive heart failure (CHF), atrial fibrillation/flutter, and CKD stage three- were extracted from VUMC's SD using a combination of ICD-9 and -10 codes, Current Procedural Terminology codes, laboratory measures, and natural-language processing. For each patient, age and BMI at the earliest time point for which a patient met case or control inclusion criteria, i.e. the decision date, is reported. All outpatient SBP and DBP collected during the six months following the decision date were obtained for each patient and individual means were calculated. History of CKD stage three was determined by an estimated glomerular filtration rate >30 mL/min/1.73m^2 and <60 mL/min/1.73m^2 calculated using the Modification of Diet in Renal Disease (MDRD) formula[128] at any point before case/control identification or within six months of identification. Patient race was administratively assigned in the SD based on either patient or physician report. Previous work has shown that self-identified race is highly correlated with genetic ancestry[129, 130] and administratively assigned race in VUMC's SD is sufficient for many genetic association analyses[131] and correlates tightly with genetic ancestry.[130]

Table 2 lists exclusion criteria for both cases and controls. Exclusion criteria included recorded ICD-9 and -10 codes for conditions known to cause secondary hypertension. For both case types as well as controls, patients were also excluded if they had left ventricular dysfunction

defined as an ejection fraction ≤ 35%. Patients with CKD stages four and five, defined by an estimated glomerular filtration rate < 30 mL/min 1.73 m^2, were also excluded.

After the algorithms were iteratively refined, a blinded review of randomly chosen, never overlapping, individual electronic medical records were performed to determine electronic algorithm efficacy. Based on a population size of 24,906, all cases and controls regardless of race, review of 138 records would allow us to estimate a misclassification rate of 10% with a margin of error of 5%. We therefore chose to review 150 records to determine algorithm efficacy. Two of the three reviewers were not affiliated with the development of the electronic algorithms. Each review drew patient records from the SD that had not been previously reviewed. The algorithms were refined until a negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity greater than 90% was achieved based on the review of 150 records subdivided equally among the two case types and controls (50 each). The final version of the algorithm is available at Phenotype KnowledgeBase (PheKB).[132]

Statistical methods

Data are presented as frequencies for categorical variables and median and interquartile range for continuous variables. Between-group comparisons were made using Pearson test for categorical variables and the Wilcoxon test for continuous variables. The false discovery rate (FDR)-adjusted p values are reported for the multiple comparisons in medication use. Analyses were conducted for the total group and also within EA or AA. Multivariable logistic regression models for medication use in resistant hypertensive cases were fit for race, age, gender, and history of T2DM, sleep apnea, IHD, TIA, atrial fibrillation/flutter, congestive heart failure with

preserved ejection fraction (HFpEF), and CKD stage three. All statistical analyses were run using the SPSS software version 24 (SPSS, Chicago, IL, USA) or R 3.3.0.[133]

2.4 Results

Algorithm validation

NPV, PPV, sensitivity, and specificity of the algorithms for resistant hypertension (Case Type I and Type II) and controlled hypertension (Control) were determined after a blinded chart review of 150 patients. The algorithm for Case Type II had the highest PPV and specificity at 96% and 98%, respectively **(Table 3)**. The NPV and sensitivity were each 100% for Case Type I and Control.

Identification of resistant hypertensive and controlled hypertensive patients

Using the occurrence of an ICD-9 or -10 code for hypertension, as well as the presence of a SBP or DBP greater than 140 or 90 mmHg prior to or at ICD-9 or -10 occurrence, we estimated the total number of hypertensive patients in VUMC's SD to be 247,420 (22.2% of the adult patients in the SD with an available blood pressure measurement), of whom 186,015 were EA (75.2%) and 33,576 were AA (13.6%). 5,024 potential EA cases and 2,139 potential AA cases were excluded for secondary causes of hypertension. After excluding secondary causes of hypertension, 16.5% of the remaining potential AA cases (n=806) and 11.4% of the potential EA cases (n=1,993) were excluded by the algorithm due to the presence of CKD stages four and five. An additional 10 AA cases and 21 EA cases were excluded because of a LVEF <35% within a year of meeting medication criteria. In total, in the SD after algorithm execution we identified 13,541 EA patients who met one or both of the case definitions for resistant hypertension and

6,933 who met the definition for controlled hypertension. We likewise identified 3,541 AA cases and 891 AA controls **(Figure 6)**. Based on these estimates we determined the prevalence of resistant hypertension among patients without CKD stages four and five in the VUMC SD to be 7.3% for EAs and 10.5% for AAs.

Demographics of the algorithm-identified cases and controls

Regardless of race, patients with resistant hypertension were significantly older, heavier, and more likely to have T2DM, sleep apnea, atrial fibrillation, a history of TIA, IHD, or CKD stage three compared to controls (**Table 4**). There were significantly more female controls than cases among EAs, but not among AAs.

Compared to EAs, AA cases and controls were significantly younger, heavier, predominately female, and had higher prevalence of T2DM at diagnosis **(Table 4)**. The prevalence of atrial fibrillation, IHD, and CKD stage three were higher in EA compared to AA cases and controls. The prevalence of HFpEF was significantly higher in AA cases compared to EA cases. The prevalence of sleep apnea, and history of TIA were not significantly different between AA and EA patients.

Blood pressure and medication use in patients with resistant hypertension and controlled hypertension

By definition, SBP and DBP were significantly higher in patients with resistant hypertension compared to those with controlled hypertension (**Table 4**). Also by definition, all patients with resistant hypertension were prescribed either a thiazide type diuretic or a CCB (amlodipine or other DHP CCB) (**Table 5**). Patients with resistant hypertension were

significantly more likely to be prescribed every class of antihypertensive compared to controls except for miscellaneous antihypertensives.

SBP and DBP were significantly higher in AA compared to EA whether within the resistant hypertensive group or within the controlled hypertensive group (**Table 4**). Among patients with resistant hypertension, AA were more likely to be prescribed the non-thiazide diuretics, CCBs, alpha-2 agonists, ACE inhibitors, and direct-acting vasodilators, minoxidil and hydralazine specifically, than EA patients and less likely to be prescribed ARBs, BBs, torsemide or renin inhibitors (**Table 5**). In multivariable logistic regression models including age, race, gender, and history of T2DM, sleep apnea, atrial fibrillation, TIA, IHD, HFpEF, and CKD stage three, race remained a significant independent predictor of torsemide ($p=0.002$), ARB ($p<0.001$), BB ($p=<0.001$), CCB ($p<0.001$), DHP CCB ($p<0.001$), alpha-2 agonist ($p<0.001$), direct-acting vasodilator ($p<0.001$), minoxidil ($p<0.001$), hydralazine ($p<0.001$), and renin inhibitor use ($p<0.001$).

Demographics of the algorithm-identified case type I and case type II resistant hypertensive patients

To better understand prescribing patterns and patient characteristics, we compared patients with resistant hypertension defined by case definition Case Type I (uncontrolled hypertension despite treatment with three classes of antihypertensive medications) versus those defined by Case Type II (hypertensive patients prescribed four or more classes of antihypertensive medications). Patients defined by Case Type II were heavier, more often male, and more likely to have T2DM, IHD, CKD stage 3, HFpEF, and sleep apnea than those defined by the Case Type I definition (**Table 6**). Among EA, Case Type II patients were more likely to

have atrial fibrillation than Case Type I patients (**Table 6**). Among AA, Case Type II patients were significantly older than Case Type I patients (**Table 6**). DBP and SBP were significantly higher in Case Type I patients than Case Type II patients (**Table 6**) in both racial groups.

Blood pressure and medication use in patients with resistant hypertension with uncontrolled blood pressure prescribed three antihypertensive medications (case type I) and patients with resistant hypertension prescribed four or more antihypertensive medications (case type II)

Consistent with the Case Type I algorithm, all patients were prescribed three simultaneous antihypertensive medication classes. All classes of medications were prescribed more frequently in patients who met the Case Type II definition versus the Case Type I definition except for thiazide diuretics and ethacrynic acid (in both EA and AA), and miscellaneous antihypertensives, amlodipine, and amiloride (in AA). In particular, spironolactone use was increased from 2.6% to 12.4% in EA and 2.8% to 12.3% in AA. Among AA, direct-acting vasodilator use was increased from 4.7% to 22.0%, and among EA from 2.8% to 13.0% (**Table 7**).

Among EA Case Type I patients the two most common combinations of three antihypertensives had two renin lowering drugs (**Figure 7**). While among AA Case Type I patients the two most common combinations had two volume dependent drugs (**Figure 7**).

Among EA Case Type II patients 3,385 patients (66.1%) were prescribed four different classes of antihypertensive medications, 1,373 (26.8%) were prescribed five, and 366 (7.1%) were prescribed six (**Figure 8**). Among AA Case Type II patients, 935 patients (63.1%) were prescribed four different classes, 416 (28.1%) were prescribed five, and 131 (8.8%) were prescribed six different classes of antihypertensive medications (**Figure 8**).

Table 8 lists the number of Case Type II patients prescribed specific medication classes at initial diagnosis and at any point following their identification. There was an increase in prescription rate for all classes when the time frame was extended to include any point in the SD following initial identification of resistant hypertension. 37.4% of EA and 41.2% of AA Case Type II patients were eventually prescribed an aldosterone antagonist. While less than half (46.6%) of EA Case Type II patients were ever prescribed an alpha-2 agonists such as clonidine, more than half (56.4%) of AA Case Type II patients were ever prescribed an alpha-2 agonist.

2.5 Discussion

We developed algorithms that successfully identified patients with resistant hypertension and easily controlled hypertension using the EHR. Electronic support has been shown to improve accuracy of clinical data acquisition and to improve control of major cardiovascular risk factors.[134] The algorithms were highly accurate with PPVs, NPVs, sensitivity, and specificity all exceeding 92% (**Table 3**). The algorithms exhibited high accuracy with PPVs, NPVs, sensitivity, and specificity measures all exceeding 92%. We found that the characteristics of patients with resistant hypertension identified through EHR were similar to those reported previously in clinical trials. We identified significant differences in the pharmacological treatment of resistant hypertension in patients of European and African ancestry.

We observed a prevalence of resistant hypertension of 7.3% and 10.6% respectively among EA and AA hypertensive patients is consistent with previous epidemiologic studies and clinical trials, which provide prevalence estimates of 8.4% to 50%.[6-8, 10-13, 101, 135] The exclusion of patients with CKD stages four and five, as well as of patients with secondary causes of hypertension likely accounts for the lower prevalence rates.[135-137] Consistent with prior studies,

the prevalence of resistant hypertension was greater among AA compared to EA, and patients with resistant hypertension were significantly older, heavier, more likely to have CKD stage three, and had a higher incidence of T2DM than patients with controlled hypertension.[5, 135-138] While the prevalence of sleep apnea was higher in patients with resistant hypertension compared to patients with controlled hypertension, the prevalence among resistant hypertensive patients in the current study, in which sleep apnea was diagnosed by ICD-9 or -10, is lower than that reported in previous studies in which sleep apnea was defined prospectively, suggesting the need for more rigorous diagnostic approaches to sleep apnea in the clinical setting.[139-141] The prevalence of CKD stage three was increased in EA compared to AA among both cases and controls. This unexpected finding likely resulted from the exclusion of glomerulonephritis, as a secondary cause of hypertension.[142, 143] In addition, a higher proportion of AA with resistant hypertension were excluded for CKD stage four and five compared to EA.[142, 144, 145]

Consistent with prior studies, the prevalence of resistant hypertension was significantly greater among AA compared to EA.[5] Also consistent with previous studies, patients with resistant hypertension were significantly older, heavier, and had a higher incidence of T2DM than patients with easily controlled hypertension.[5] While the prevalence of sleep apnea was higher in patients with resistant hypertension compared to patients with easily controlled hypertension, the prevalence among resistant hypertensive patients in the current study is lower than that reported in previous studies in which sleep apnea was defined prospectively.[139-141] Since this study relied on retrospective identification based on the presence of a sleep apnea ICD-9 or -10 code in a patient's EHR the lower prevalence may reflect under-diagnosis of sleep apnea by clinicians. Considering that sleep apnea is a common risk factor for resistant

hypertension[5] this finding suggests the need for more rigorous diagnostic approaches to sleep apnea in the clinical setting.

Consistent with previous studies, the prevalence of CKD stage three was significantly greater in resistant hypertensive cases than easily controlled hypertensive controls regardless of race.[135-137] The prevalence of CKD stage three was significantly greater in EA than AA in both cases and controls. This unexpected finding may result from the exclusion of a higher proportion of AA with resistant hypertension for CKD stage four and five compared to EA. The higher rates of severe kidney disease, i.e. CKD stage four and five, in AA compared to EA is consistent with previous studies.[142, 144, 145] Several studies have also shown that AA with renal disease have faster declines in renal function than EA,[146, 147] which may also have contributed to the lower CKD stage three rates in AA than EA. Exclusion of glomerulonephritis as a secondary cause of hypertension from the resistant hypertension population may also contribute to the lower rates of CKD three in AA than EA in our population as AA are at an increased risk for glomerulonephritis, an established risk factor for CKD.[142, 143]

Importantly, we found that prescribing trends differed in EA and AA patients with resistant hypertension. AA patients were more likely to be treated with direct-acting vasodilators hydralazine and minoxidil and less likely to receive an ARB or renin inhibitor compared to EA. The prevalence of salt-sensitive hypertension is increased in AA compared to EA patients with hypertension, and thiazide-type diuretics and vasodilators are most effective in salt-sensitive hypertension.[148-150] Hydralazine has also been shown to reduce mortality in AA treated for heart failure[151] and awareness of this may account for increased use.[152, 153] The lower use of ARBs and renin inhibitors in AA may reflect clinician awareness of reduced efficacy of drugs that interrupt the renin-angiotensin-aldosterone system (RAAS) in studies of AA.[154-156] Thiazide diuretics or

DHP CCBs, used by definition in all patients classified as having resistant hypertension, enhance the response to RAAS interrupting drugs in AA, however.[157] Similarly aliskiren may decrease blood pressure in patients with resistant hypertensive who do not respond to spironolactone.[158] For these reasons, the decreased use of ARBs and renin inhibitors in AA with resistant hypertension prescribed a thiazide diuretic or DHP CCB is surprising. Whether differences in patterns of drug treatment in AA and EA patients with resistant hypertension reflect personalized prescribing or prescribing bias requires further study.

We also evaluated trends in the escalation of antihypertensive treatment in resistant hypertension by comparing medication use between Case Types I and II. The efficacy of spironolactone as an add-on therapy for blood pressure lowering in patients with resistant hypertension has been supported by many studies and is suggested as a fourth line treatment by various international guidelines.[48, 51, 159-163] In the present clinical population aldosterone antagonist use increased with the addition of a fourth med with a prescription rate of approximately 3% in Case Type I patients compared to 12% in Case Type II patients, regardless of race. With extended follow-up of patients who met the Case Type II definition, use of an aldosterone antagonist increased to 37.4% in EA and to 41.2% in AA.

The identification of the resistant hypertensive population using the EHR is not without limitations. First, patients may not adhere to prescribed medication. The true prevalence of medication non-adherence in the resistant hypertensive population is unknown and the estimates from various studies range from as low as 16% up to 53%.[54-57] While we could confirm that resistant hypertensive patients were prescribed three or more antihypertensive medication classes simultaneously in their EHR, without directly measuring the medication or its metabolites in a patient's plasma or urine we are unable to confirm adherence. Using a pharmacy fill rate of

<80% to exclude patients who were non-adherent with antihypertensive medications, Pimienta et al. reported an incidence of true resistant hypertension of 1.9%.[164] We recently reported a rigorous adherence rate of 58.8%, among hypertensive patients in an emergency department prescribed three or more antihypertensive medications based on the detection of drugs in the plasma.[165] Second, we used outpatient office blood pressure measurements to define resistant hypertension in the EHR, but ambulatory measurements would be necessary to distinguish between apparent resistant and true resistant hypertension.[10] Lastly, it is possible that offsite prescriptions or discontinuations of antihypertensive medications were not captured in the EHR; we overcame this potential limitation by requiring repeated documentation of a medication over more than a month in the study algorithms. Nevertheless, in summary, we demonstrate the feasibility of identifying a large number of patients with resistant hypertension and controlled hypertension using an EHR. Using the methodology we replicated findings previously reported in population studies,[5, 12, 166] and identified differing patterns of antihypertensive medication use in AA and EA with resistant hypertension. Future research using these algorithms has the potential to provide larger patient populations than have been studied previously for the evaluation of outcome studies as well as genetic associations in systems where the electronic health records are linked to DNA.

2.6 Conclusions

We demonstrate the feasibility of identifying patients with resistant hypertension and easily controlled hypertension using an EHR. More importantly, we identified trends in the prescribing of different antihypertensive classes to AA and EA with resistant hypertension in an academic medical center. Our findings replicate many of the clinical characteristics of these

populations previously reported in population studies.[5, 12] This replication of specific characteristics of the resistant hypertensive phenotype in the EHR identified population supports the use of this population for future studies, specifically genetic association studies to potentially identify novel drivers of the phenotype.

2.7 Figures

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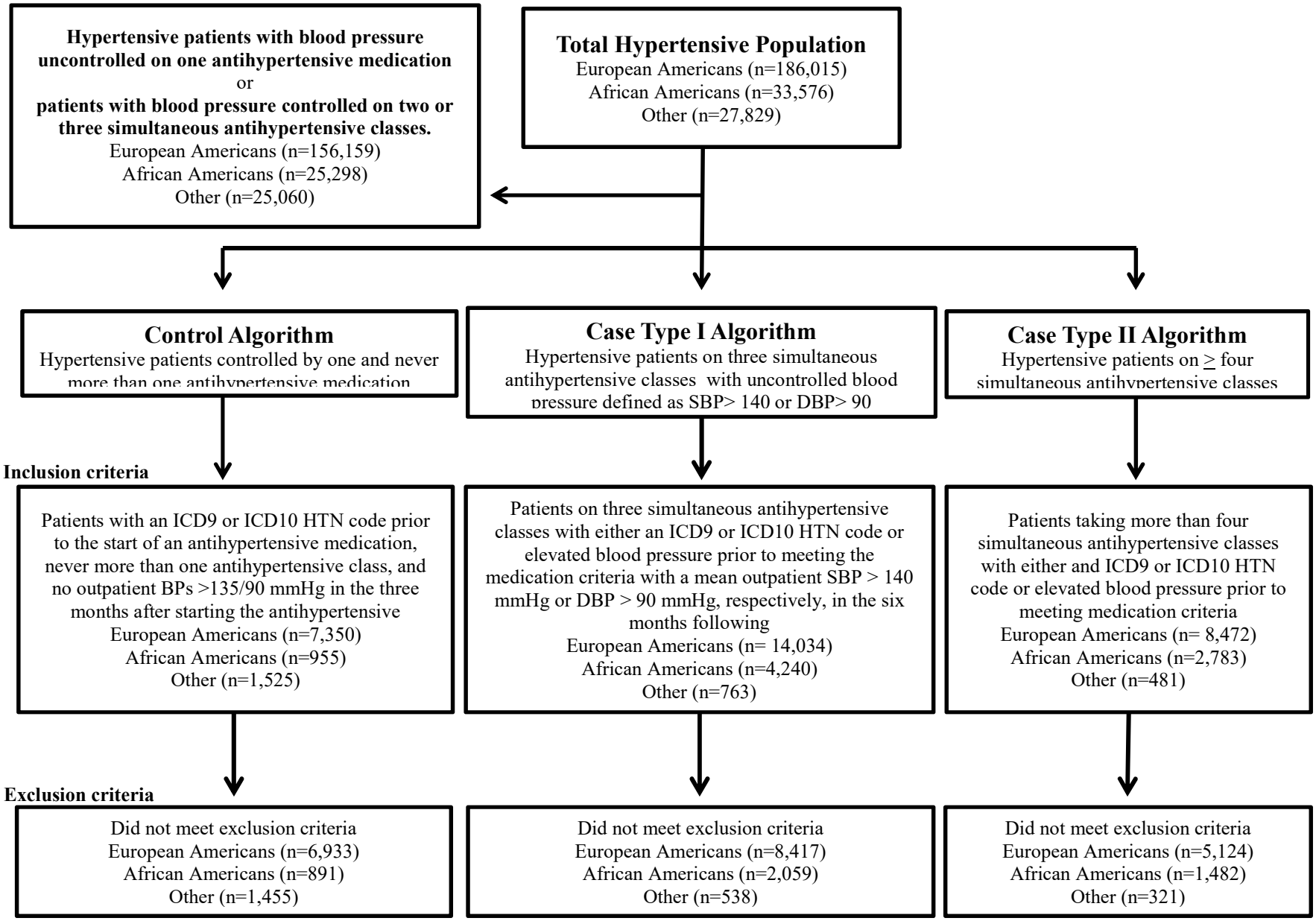

Figure 6. Diagram of the algorithms for the identification of patients with resistant (cases) and easily controlled (control) hypertension in Vanderbilt University Medical Center's synthetic derivative of the electronic health record

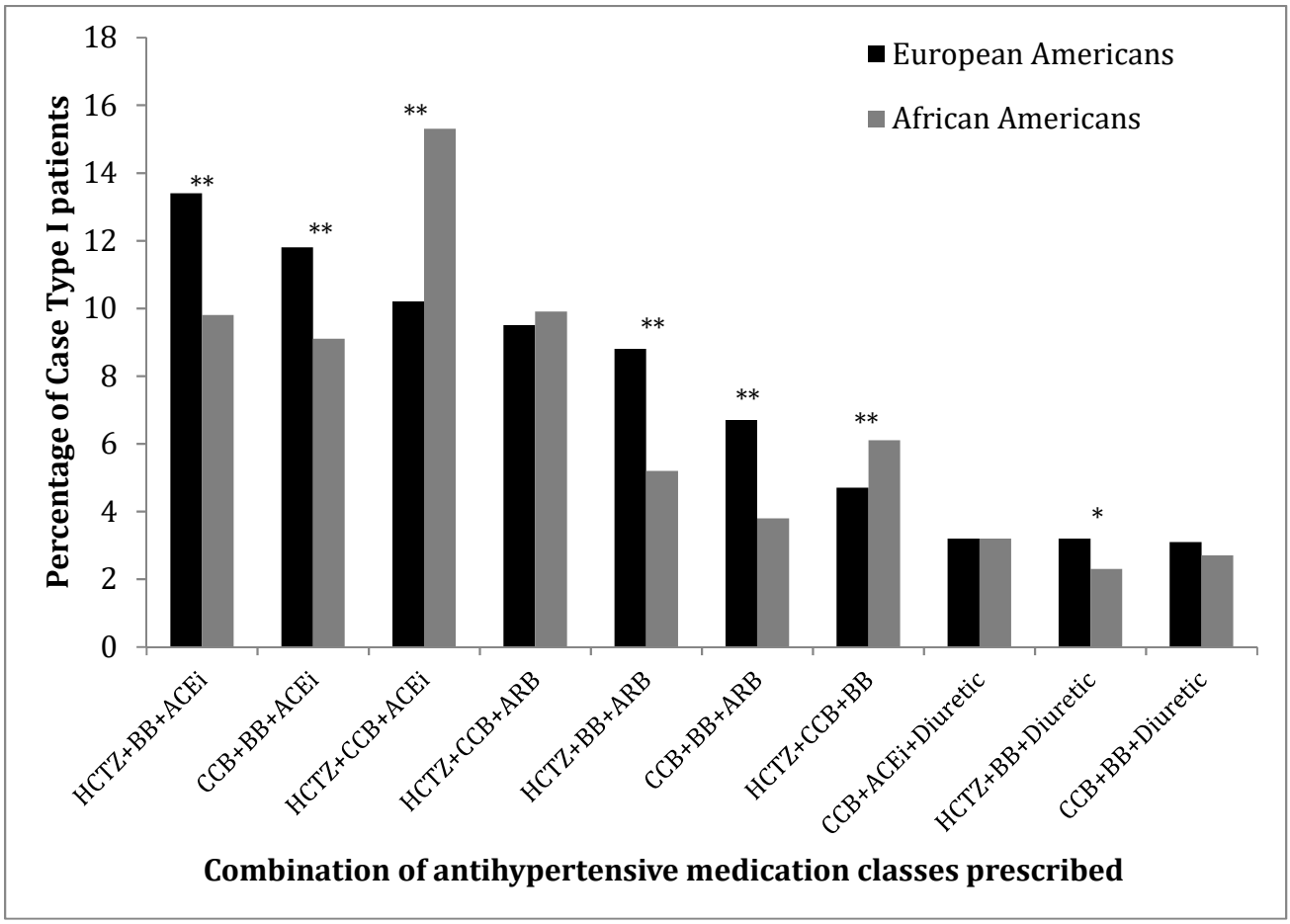

Figure 7. The percentage of Case Type I patients prescribed a specific combination of antihypertensive medications based on class stratified by race. Antihypertensive medication groups are as follows: ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; BB, beta blockers; CCB, calcium channel blockers including both the dihydropyridine and nondihydropyridine classes; Diuretic, includes diuretics that do not belong to the thiazide class such as K-sparing and loop diuretics; HCTZ, includes thiazide diuretics, such as hydrochlorothiazide, and thiazide-like diuretics. * denotes a p-values < 0.05 and ** denotes a p-value < 0.001 based on a Pearson's chi-squared test.

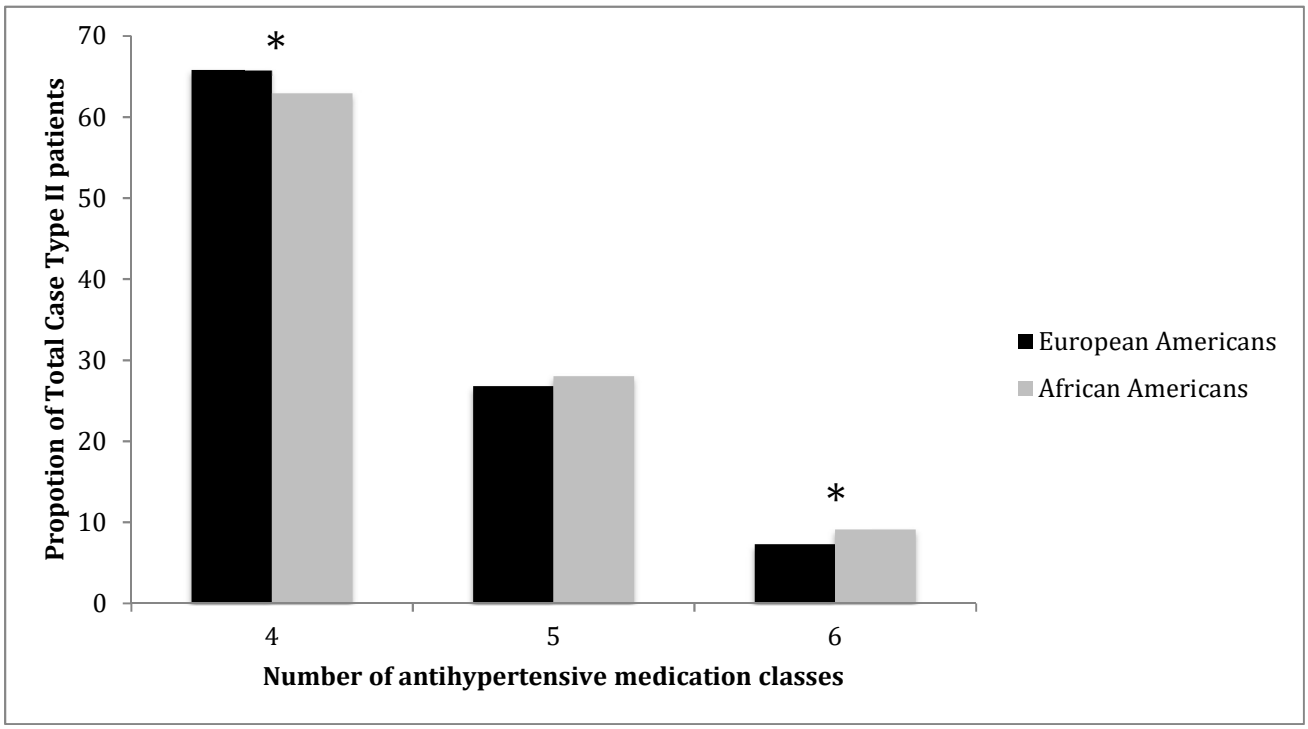

Figure 8. The percentage of Case Type II patients prescribed four, five, or six simultaneous antihypertensive medication classes at diagnosis based on race. * denotes a p-value of <0.05 based on Pearson's chi-squared test.

2.8 Tables

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Table 1. Resistant hypertension algorithm antihypertensive classes and medications

Medication Class	Medication Names
Aldosterone antagonist	eplerenone, spironolactone, inspra, aldactone
ACEi	fosinopril, fosinopril sodium, ramipril, captopril, moexipril, lisinopril, enalapril, quinapril, trandolapril, benazepril, perindopril, monopril, altace, capoten, univasc, zestril, prinivil, vasotec, epaned, accupril, mavik, gopten, odrik, lotensin, aceon
ARB	eprosartan, azilsartan medoxomil, olmesartan, valsartan, telmisartan, losartan, candesartan, irbesartan, teveten, edarbi, benicar, diovan, micardis, cozaar, atacand, avapro
ARB + Renin inhibitor	valsartan/aliskiren, valturna
ARB + Neprilysin inhibitor	valsartan/sacubitril, entresto
Alpha-2 agonist	clonidine, kapvay, catapres, duraclon, jenloga, nexiclon, guanfacine, methyldopa, methyldopate, guanabenz, tenex, intuniv, aldomet, wytensin
Alpha antagonist	prazosin, prazosin hydrochloride, minipress, terazosin, terazosin hydrochloride, doxazosin, vasoflex, lentopres, hypovase, hytrin, cardura
BB	betaxolol, kerlone, betoptic, acebutolol, sectral, atenolol, tenormin, metoprolol, metoprolol succinate, troprol, metoprolol tartrate, lopressor, dutoprolol, nebivolol, bystolic, bisoprolol, zebeta, esmolol, brevibloc, propranolol, innopran, inderal, nadolol, corgard, coreg, labetalol, timolol, carteolol, penbutolol, pindolol, normodyne, trandate, blocadren, timoptic, istalol, betimol, cartrol, levatol, visken
BB + Diuretic	pindolol/clopamide, viskalidix
CCB	nifedipine, diltiazem, verapamil, isradipine, felodipine, amlodipine, nicardipine, nisoldipine, bepridil, clevidipine, afeditab, adalat, nifediac, procardia, nifedical, dilt, diltia, cartia, cardizem, taztia, dilacor, diltzac, matzim, tiazac, verlan, calan, isoptin, covera, dynacirc, plendil, norvasc, cardene, sular, vascor, cleviprex
CCB + Renin inhibitor	amlodipine/aliskiren, tekamlo
CCB + Statin	amlodipine/atorvastatin, caduet
Loop diuretic	bumetanide, bumex, ethacrynic acid, ethacrynate, edecrin, sodium edicrin, torsemide, demadex, furosemide, lasix
Potassium-sparing diuretic	triamterene, dyrenium, spironolactone, aldactone, amiloride, midamor

Combination diuretic	furosemide/amiloride, frumil
Thiazide diuretic	Indapamide, lozol, natrilix, thalitone, chlorthalidone, metolazone, zaroxolyn, mydrox, hydrochlorothiazide, hctz, hct, hydrodiuril, aquazide, esidrix, microzide, methylchlorothiazide, aquatense, enduron, chlorothiazide, diuril, diuril sodium, bendroflumethiazide, bendrofluazide, aprinox, polythiazide, renese, hydroflumethiazide, saluron, cyclothiazide, benzthiazide
Renin inhibitor	aliskiren, tekturna
Vasodilator	hydralazine, minoxidil, apresazide, apresoline, loniten, bidil, hydralazine/isosorbide dinitrate
Misc antihtn	reserpine, raudixin, serpalan, serpasil
Vasodilator + HCTZ	hydralazine/hydrochlorothiazide, hydra-zide, apresazide
Renin inhibitor + HCTZ	aliskiren/hydrochlorothiazide, tekturna hct
Aldosterone antagonist + HCTZ	spironolactone/hydrocholorothiazide, aldactazide
Alpha antagonist + HCTZ	prazosin/polythiazide, minizide
ACEi + HCTZ	fosinopril/hydrochlorothiazide, captopril/hydrochlorothiazide, moexipril/hydrochlorothiazide, lisinopril/hydrochlorothiazide, enalapril/hydrochlorothiazide, quinapril/hydrochlorothiazide, benazepril/hydrochlorothiazide, monopril hct, capozide, uniretic, prinivil, zestoretic, prinzide, vaseretic, quinaretic, accuretic, lotensin hct
ARB +HCTZ	eprosartan/hydrochlorothiazide, azilsartan/chlorthalidone, candesartan/hydrochlorothiazide, irbesartan/hydrochlorothiazide, losartan/hydrochlorothiazide, telmisartan/hydrochlorothiazide, tevetan hct, edarbyclor, benicar hct, diovan hct, atacand hct, avalide, hyzaar, micardis hct
Alpha-2 agonist + HCTZ	clonidine/chlorthalidone, clorpres, combipress, aldoril, methyldopa/hydrochlorothiazide
BB + HCTZ	acebutolol/hydrochlorothiazide, atenolol/hydrochlorothiazide, metoprolol/hydrochlorothiazide, bisoprolol/hydrochlorothiazide, propranolol/hydrochlorothiazide, nadolol/hydrochlorothiazide, timolol/hydrochlorothiazide, pindolol/hydrochlorothiazide, tenoretic, lopressor hct, dutoprol, ziac, inderide, corzide, timolide, viskazide

Diuretic + HCTZ	triamterene/hydrochlorothiazide, amiloride/hydrocholorthiazide, triamterene/benzthiazide, dyazide, maxzide, moduretic, ditide
	enalapril/felodipine, trandolapril/verapamil, benazepril/amlodipine, lexxel, tarka, lotrel, amlobenz, prestalia, perindopril/amlodipine
ACEi + CCB	lexxel, tarka, lotrel, amlobenz, prestalia, enalapril/felodipine, trandolapril/verapamil, benazepril/amlodipine, perindopril/amlodipine
ARB + CCB	olmesartan/amlodipine, valsartan/amlodipine, telmisartan/amlodipine, azor, exforge, twynsta
Reserpine + Vasodilator +HCTZ	reserpine/hydralazine/hydrochlorothiazide, Ser-Ap-Es
Renin inhibitor + CCB +HCTZ	aliskiren/amlodipine/hydrochlorothiazide, tribenzor
ARB + CCB + HCTZ	olmesartan/amlodipine/hydrochlorothiazide, valsartan/amlodipine/hydrocholorothiazide, tribenzor, exforge hct

Abbreviations: ARB, angiotensin receptor blocker; ACEi, angiotensin converting enzyme inhibitor; BB, Beta blocker; CCB, calcium channel blocker; HCTZ, hydrochlorothiazide; misc antihtn, miscellaneous antihypertensive

Table 2. **ICD-9 and -10 exclusions for case and control algorithm definitions**

Description	ICD-9-CM codes	ICD-10-CM-codes
Exclusion for Case Type I, Case Type II, and Control if ever present in a patient record		
Malignant neoplasm of unspecified adrenal gland	194.0	C74.9
Benign neoplasm of unspecified adrenal gland	227.0	D35.00
Disorders of the adrenal gland (excluding adrenal insufficiencies)	255.0, 255.1*, 255.2, 255.3, 255.6, 255.8, 255.9	E24.*, E26.*, E25.*, E27.0, E27.5, E27.8, E27.9
Secondary Hypertension	405.*	I15.0, I15.8
Chronic pulmonary heart disease	416.*	I27. *
Nephrotic syndrome	581.*	N04.*, N08
Chronic glomerulonephritis	582.*	N03.*, N08
Bulbus cordis anomalies	745.*	Q20.*, Q21.*
Coarctation of aorta	747.1*	Q25.1, Q25.2
Exclusion for Case Type I and Case Type II if present in a patient record 5 years before or 1 year after identification as a Case		
Thyrotoxicosis	242.*	E05.*
Disorder of thyrocalcitonin secretion	246.0	E07.0
Disorders of the thyroid NEC	246.8	E03.4, E07.89
Disorders of the thyroid NOS	246.9	E07.9
Parathyroid disorder NEC	252.8	E21.4
Parathyroid disorder NOS	252.9	E21.5
Obstructive uropathy	599.6*	N13.9

Abbreviations: NEC, not elsewhere classified; NOS, not otherwise classified

Table 3. **Algorithm validation metrics following blinded chart review**

	Resistant Hypertension		
	Case Type I	Case Type II	Control
Positive predictive value	94% (83%-98%)	96% (85%-99%)	92% (80%-97%)
Negative predictive value	100% (95%-100%)	99% (94%-100%)	100% (95%-100%)
Specificity	97% (91%-99%)	98% (92%-100%)	96% (90%-99%)
Sensitivity	100% (91%-100%)	98% (88%-100%)	100% (90%-100%)

Data are presented as % (95% Confidence Interval)

Table 4. Characteristics of European American and African American patients with resistant hypertension (cases) or easily controlled hypertension (controls)

	European Americans (EA)			African Americans (AA)			EA vs AA	
Variable	**Cases (n=13541)**	**Controls (n=6933)**	**p-value**	**Cases (n=3541)**	**Controls (n=891)**	**p-value**	**Cases p-value**	**Controls p-value**
SBP, mmHg	145.0 (140.0-153.0)	120.8 (114.0-127.0)	<0.001	147.4 (141.0-156.0)	122.0 (115.0-128.0)	<0.001	<0.001	0.001
DBP, mmHg	78.0 (70.0-86.0)	74.0 (68.0-80.0)	<0.001	85.0 (76.4-92.2)	75.5 (70.0-81.0)	<0.001	<0.001	<0.001
Age, years	66.0 (57.0-73.0)	53.0 (43.0-64.0)	<0.001	56.0 (47.0-65.0)	46.0 (34.0-55.0)	<0.001	<0.001	<0.001
BMI, kg/m^2	30.8 (26.7-35.8)	29.3 (25.4-34.2)	<0.001	32.9 (28.3-38.9)	31.0 (26.3-37.0)	<0.001	<0.001	<0.001
Female, n (%)	6615 (48.9%)	3495 (50.4%)	0.04	2092 (59.1%)	527 (59.2%)	0.97	<0.001	<0.001
T2DM, n (%)	2694 (19.9%)	1026 (14.8%)	<0.001	954 (26.9%)	171 (19.2%)	<0.001	<0.001	<0.001
Sleep Apnea, n (%)	868 (6.4%)	373 (5.4%)	0.003	252 (7.1%)	45 (5.1%)	0.03	0.13	0.68
Afib, n (%)	1424 (10.5%)	272 (3.9%)	<0.001	130 (3.7%)	11 (1.2%)	<0.001	<0.001	<0.001
TIA, n (%)	603 (4.5%)	110 (1.6%)	<0.001	176 (5.0%)	19 (2.1%)	<0.001	0.19	0.23
IHD, n (%)	2493 (18.4%)	585 (8.4%)	<0.001	468 (13.2%)	32 (3.6%)	<0.001	<0.001	<0.001
CKD 3, n (%)*	4407 (42.4%)	650 (14.1%)	<0.001	870 (29.7%)	47 (7.2%)	<0.001	<0.001	<0.001
HFpEF, n (%)	1173 (9%)	102 (1%)	<0.001	376 (11%)	16 (2%)	<0.001	<0.001	0.454

* The percentage for CKD 3 is based on the number of subjects with available estimated glomerular filtration rate (eGFR) data, not all subjects in the population. The number of subjects with available eGFR data is: 10,405 EA Cases; 4,602 EA Controls; 2,927 AA Cases; and 653 AA Controls.

Data are presented as median (interquartile range) unless otherwise indicated.

Abbreviations: Afib, atrial fibrillation; BMI, body mass index; CKD 3, chronic kidney disease stage 3; DBP, diastolic blood pressure; HFpEF, congestive heart failure with preserved ejection fraction; IHD, ischemic heart disease; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TIA, transient ischemic attack

Table 5. Medication use in European American and African American patients with resistant hypertension (cases) and easily controlled hypertension (controls)

	European Americans (EA)			African Americans (AA)			EA vs AA	
Variable	**Case (n=13541)**	**Control (n=6933)**	**p-value**	**Case (n=3541)**	**Control (n=891)**	**p-value**	**Case p-value**	**Control p-value**
Thiazide/CCB, n (%)	13541 (100%)	1167 (16.8%)	<0.001	3541 (100%)	292 (32.8%)	<0.001	1.0	<0.001
ACE inhibitor, n (%)	6999 (51.7%)	2439 (35.2%)	<0.001	1916 (54.1%)	264 (29.6%)	<0.001	0.02	0.003
ARB, n (%)	5178 (38.2%)	728 (10.5%)	<0.001	1161 (32.8%)	54 (6.1%)	<0.001	<0.001	<0.001
BB, n (%)	8697 (64.2%)	1803 (26.0%)	<0.001	1996 (56.4%)	152 (17.1%)	<0.001	<0.001	<0.001
Alpha-2 agonist, n (%)	1921 (14.2%)	140 (2.0%)	<0.001	736 (20.8%)	29 (3.3%)	<0.001	<0.001	0.04
CCB, n (%)	9272 (68.5%)	615 (8.9%)	<0.001	2625 (74.1%)	144 (16.2%)	<0.001	<0.001	<0.001
Amlodipine, n (%)	6759 (49.9%)	318 (4.6%)	<0.001	1749 (49.4%)	90 (10.1%)	<0.001	0.74	<0.001
DHP CCB, n (%)	1508 (11.1%)	75 (1.1%)	<0.001	585 (16.5%)	27 (3.0%)	<0.001	<0.001	<0.001
Non-DHP CCB, n (%)	1005 (7.4%)	222 (3.2%)	<0.001	291 (8.2%)	27 (3.0%)	<0.001	0.18	0.84
Thiazide Diuretic, n (%)	8812 (65.1%)	774 (11.2%)	<0.001	2319 (65.5%)	175 (19.6%)	<0.001	0.76	<0.001
Aldosterone antagonist, n (%)	854 (6.3%)	57 (0.8%)	<0.001	240 (6.8%)	8 (0.9%)	<0.001	0.43	0.85
Non-thiazide Diuretic, n (%)	4271 (31.5%)	326 (4.7%)	<0.001	1190 (33.6%)	56 (6.3%)	<0.001	0.04	0.08
Furosemide, n (%)	3114 (23.0%)	254 (3.7%)	<0.001	874 (24.7%)	44 (4.9%)	<0.001	0.07	0.10
Triamterene, n (%)	1155 (8.5%)	45 (0.7%)	<0.001	352 (9.9%)	11 (1.2%)	<0.001	0.02	0.09
Torsemide, n (%)	176 (1.3%)	17 (0.2%)	<0.001	23 (0.7%)	1 (0.1%)	0.06	0.004	0.57
Bumetanide, n (%)	141 (1.0%)	8 (0.1%)	<0.001	28 (0.8%)	0 (0.0%)	0.01	0.28	0.43
Amiloride, n (%)	32 (0.2%)	1 (0.0%)	<0.001	10 (0.3%)	0 (0.0%)	0.12	0.75	0.79
Ethacrynic acid, n (%)	11 (0.1%)	1 (0.0%)	0.07	7 (0.2%)	0 (0.0%)	0.19	0.10	0.79
Vasodilator, n (%)	903 (6.7%)	17 (0.3%)	<0.001	422 (11.9%)	2 (0.2%)	<0.001	<0.001	0.93

Minoxidil, n (%)	161 (1.2%)	2 (0.0%)	<0.001	101 (2.9%)	0 (0.0%)	<0.001	<0.001	0.75
Hydralazine, n (%)	742 (5.5%)	15 (0.2%)	<0.001	321 (9.1%)	2 (0.2%)	<0.001	<0.001	0.96
Alpha antagonist, n (%)	626 (4.6%)	33 (0.5%)	<0.001	139 (3.9%)	7 (0.8%)	<0.001	0.12	0.33
Renin inhibitor, n (%)	275 (2.0%)	1 (0.0%)	<0.001	43 (1.2%)	0 (0.0%)	0.001	0.004	0.79
Misc antihtn, n (%)	3 (0.0%)	0 (0.0%)	0.21	0 (0.0%)	0 (0.0%)	1.0	0.51	1.0

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BB, beta blocker; CCB, calcium channel blocker; DHP, dihydropyridine; Misc antihtn, miscellaneous antihypertensive

p-values were adjusted to control for false discovery rate

Table 6. Demographics of European American and African American patients with resistant hypertension stratified by case type

Variable	European Americans (EA)			African Americans (AA)		
	Case Type I (n=8417)	Case Type II (n=5124)	p-value	Case Type I (n=2059)	Case Type II (n=1482)	p-value
SBP, mmHb	147.0 (142.1-154.0)	137.0 (126.0-148.7)	<0.001	148.7 (143.0-156.4)	144.2 (132.0-154.8)	<0.001
DBP, mmHg	80.0 (72.2-88.3)	73.2 (66.0-80.7)	<0.001	87.0 (78.8-93.0)	80.7 (73.0-89.8)	<0.001
Age, years	66.0 (57.0-73.0)	66.0 (57.0-73.0)	0.40	56.0 (47.0-65.0)	57.0 (48.0-66.0)	0.03
BMI, kg/m^2	30.3 (26.4-35.3)	31.5 (27.4-36.5)	<0.001	32.5 (27.9-38.2)	33.7 (28.9-40.3)	<0.001
Female, n (%)	4215 (50.1%)	2400 (46.8%)	<0.001	1249 (60.7%)	843 (56.9%)	0.02
T2DM, n (%)	1140 (13.5%)	1554 (30.3%)	<0.001	343 (16.7%)	611 (41.2%)	<0.001
Sleep Apnea, n (%)	394 (4.7%)	474 (9.3%)	<0.001	94 (4.6%)	158 (10.7%)	<0.001
Afib, n (%)	794 (9.4%)	630 (12.3%)	<0.001	66 (3.2%)	64 (4.3%)	0.08
TIA, n (%)	375 (4.5%)	228 (4.5%)	0.99	110 (5.3%)	66 (4.5%)	0.23
IHD, n (%)	1417 (16.8%)	1076 (21.0%)	<0.001	229 (11.1%)	239 (16.1%)	<0.001
CKD 3, n (%)*	2602 (38.7%)	1805 (49.0%)	<0.001	466 (25.7%)	404 (36.3%)	<0.001

* The percentage for CKD 3 is based on the number of subjects with available estimated glomerular filtration rate (eGFR) data, not all subjects in the population. The number of subjects with available eGFR data is: 6,719 EA Case Type I; 3,686 EA Case Type II; 2,927 AA Cases; and 653 AA Controls.

Data are presented as median (interquartile range) unless otherwise indicated.

Abbreviations: Afib, atrial fibrillation; BMI, body mass index; CKD 3, chronic kidney disease stage three; DBP, diastolic blood pressure; IHD, ischemic heart disease; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TIA, transient ischemic attack

Table 7. Medication use in European American and African American patients with resistant hypertension based on case type definition

	European Americans (EA)			African Americans (AA)			EA vs AA	
Variable	**Case I (n=8417)**	**Case II (n=5124)**	**p-value**	**Case I (n=2059)**	**Case II (n=1482)**	**p-value**	**Case I p-value**	**Case II p-value**
Thiazide/CCB, n (%)	8417 (100%)	5124 (100%)	1.0	2059 (100%)	1482 (100%)	1.0	1.0	1.0
ACE inhibitor, n (%)	4051 (48.1%)	2948 (57.5%)	<0.001	1037 (50.4%)	879 (59.3%)	<0.001	0.14	0.32
ARB, n (%)	2861 (34.0%)	2317 (45.2%)	<0.001	571 (27.7%)	590 (39.8%)	<0.001	<0.001	<0.001
BB, n (%)	4718 (56.1%)	3979 (77.7%)	<0.001	934 (45.4%)	1062 (71.7%)	<0.001	<0.001	<0.001
Alpha-2 agonist, n (%)	544 (6.5%)	1377 (26.9%)	<0.001	237 (11.5%)	499 (33.7%)	<0.001	<0.001	<0.001
CCB, n (%)	5297 (62.9%)	3975 (77.6%)	<0.001	1433 (69.6%)	1192 (80.4%)	<0.001	<0.001	0.05
Amlodipine, n (%)	4027 (47.8%)	2732 (53.3%)	<0.001	1000 (48.6%)	749 (50.5%)	0.26	0.64	0.13
DHP CCB, n (%)	752 (8.9%)	756 (14.8%)	<0.001	293 (14.2%)	292 (19.7%)	<0.001	<0.001	<0.001
Non-DHP CCB, n (%)	518 (6.2%)	487 (9.5%)	<0.001	140 (6.8%)	151 (10.2%)	<0.001	0.39	0.52
Thiazide Diuretic, n (%)	5448 (64.7%)	3364 (65.7%)	0.28	1363 (66.2%)	956 (64.5%)	0.30	0.31	0.52
Aldosterone antagonist, n (%)	219 (2.6%)	635 (12.4%)	<0.001	58 (2.8%)	182 (12.3%)	<0.001	0.64	0.91
Non-thiazide Diuretic, n (%)	1643 (19.5%)	2628 (51.3%)	<0.001	397 (19.3%)	793 (53.5%)	<0.001	0.84	0.24
Furosemide, n (%)	1074 (12.8%)	2040 (39.8%)	<0.001	241 (11.7%)	633 (42.7%)	<0.001	0.31	0.11
Triamterene, n (%)	599 (7.1%)	556 (10.9%)	<0.001	171 (8.3%)	181 (12.2%)	<0.001	0.13	0.25
Torsemide, n (%)	43 (0.55)	133 (2.6%)	<0.001	4 (0.2%)	19 (1.3%)	<0.001	0.12	0.01
Bumetanide, n (%)	47 (0.6%)	94 (1.8%)	<0.001	8 (0.4%)	20 (1.3%)	0.002	0.46	0.31
Amiloride, n (%)	5 (0.15)	27 (0.5%)	<0.001	3 (0.1%)	7 (0.5%)	0.08	0.31	0.84
Ethacrynic acid, n (%)	4 (0.0%)	7 (0.1%)	0.09	2 (0.1%)	5 (0.3%)	0.12	0.51	0.21
Vasodilator, n (%)	236 (2.8%)	667 (13.0%)	<0.001	96 (4.7%)	326 (22.0%)	<0.001	<0.001	<0.001
Minoxidil, n (%)	27 (0.3%)	134 (2.6%)	<0.001	7 (0.3%)	94 (6.3%)	<0.001	0.91	<0.001
Hydralazine, n (%)	209 (2.5%)	533 (10.4%)	<0.001	89 (4.3%)	232 (15.7%)	<0.001	<0.001	<0.001
Alpha antagonist, n (%)	159 (1.9%)	467 (9.1%)	<0.001	35 (1.7%)	104 (7.0%)	<0.001	0.64	0.03

Renin inhibitor, n (%)	58 (0.7%)	217 (4.2%)	<0.001	11 (0.5%)	32 (2.2%)	<0.001	0.52	<0.001
Misc antihtn, n (%)	0 (0.0%)	3 (0.1%)	0.03	0 (0.0%)	0 (0.0%)	1.0	1.0	0.47

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BB, beta blocker; CCB, calcium channel blocker; DHP, dihydropyridine; Misc antihtn, miscellaneous antihypertensive

p-values were adjusted to control for false discovery rate

Table 8: Medications prescribed to Case Type II patients with resistant hypertension at initial diagnosis (initial therapies) compared to medications prescribed at any point in the electronic health record after initial diagnosis (ever prescribed)

	European Americans (n=5124)		African Americans (n=1482)	
Variable	**Initial therapies**	**Ever prescribed**	**Initial therapies**	**Ever prescribed**
Thiazide/CCB, n (%)	5124 (100%)	5124 (100%)	1482 (100%)	1482 (100%)
ACE inhibitor, n (%)	2948 (57.5%)	3726 (72.7%)	879 (59.3%)	1143 (77.1%)
ARB, n (%)	2317 (45.2%)	3311 (64.6%)	590 (39.8%)	935 (63.1%)
BB, n (%)	3979 (77.7%)	4872 (95.1%)	1062 (71.7%)	1395 (94.1%)
Alpha-2 agonist, n (%)	1377 (26.9%)	2386 (46.6%)	499 (33.7%)	836 (56.4%)
CCB, n (%)	3975 (77.6%)	4743 (92.6%)	1192 (80.4%)	1420 (95.8%)
Amlodipine, n (%)	2732 (53.3%)	3837 (74.9%)	749 (50.5%)	1122 (75.7%)
DHP CCB, n (%)	756 (14.8%)	1626 (31.7%)	292 (19.7%)	614 (41.4%)
Non-DHP CCB, n (%)	487 (9.5%)	1313 (25.6%)	151 (10.2%)	371 (25.0%)
Thiazide Diuretic, n (%)	3364 (65.7%)	4288 (83.7%)	956 (64.5%)	1236 (83.4%)
Aldosterone antagonist, n (%)	635 (12.4%)	1916 (37.4%)	182 (12.3%)	610 (41.2%)
Non-thiazide Diuretic, n (%)	2628 (51.3%)	4138 (80.8%)	793 (53.5%)	1183 (79.8%)
Furosemide, n (%)	2040 (39.8%)	3611 (70.5%)	633 (42.7%)	1026 (69.2%)
Triamterene, n (%)	556 (10.9%)	745 (14.5%)	181 (12.2%)	251 (16.9%)
Torsemide, n (%)	133 (2.6%)	384 (7.5%)	19 (1.3%)	84 (5.7%)
Bumetanide, n (%)	94 (1.8%)	338 (6.6%)	20 (1.3%)	90 (6.1%)
Amiloride, n (%)	27 (0.5%)	65 (1.3%)	7 (0.5%)	26 (1.8%)
Ethacrynic acid, n (%)	7 (0.1%)	29 (0.6%)	5 (0.3%)	7 (0.5%)
Vasodilator, n (%)	667 (13.0%)	1925 (37.6%)	326 (22.0%)	761 (51.4%)
Minoxidil, n (%)	134 (2.6%)	359 (7.0%)	94 (6.3%)	207 (14.0%)

Hydralazine, n (%)	533 (10.4%)	1706 (33.3%)	232 (15.7%)	664 (44.8%)
Alpha antagonist, n (%)	467 (9.1%)	807 (15.8%)	104 (7.0%)	209 (14.1%)
Renin inhibitor, n (%)	217 (4.2%)	386 (7.5%)	32 (2.2%)	65 (4.4%)
Misc antihtn, n (%)	3 (0.1%)	4 (0.1%)	0 (0.0%)	0 (0.0%)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BB, beta blocker; CCB, calcium channel blocker; DHP, dihydropyridine; Misc antihtn, miscellaneous antihypertensive

CHAPTER 3

ASSOCIATION OF *CYP4A11* LOSS-OF-FUNCTION VARIANTS AND RESISTANT HYPERTENSION

3.1 Abstract

Excessive dietary salt intake is common in patients with hypertension in the United States and salt-sensitivity contributes to the pathophysiology of resistant hypertension. In Dahl salt-sensitive rats, 20-hydroxyeicosatetraenoic acid (20-HETE) deficiency contributes to the development of salt-sensitive hypertension. In humans, dietary salt intake regulates the excretion of 20-HETE and patients with salt-sensitive hypertension have a disrupted relationship between 20-HETE and sodium excretion, e.g. sodium excretion does not correlate with endogenous 20-HETE levels. We, therefore, hypothesized that rs1126742, a loss-of-function variant in the gene *CYP4A11* encoding the enzyme responsible for the formation of 20-HETE in humans, as well as a more common variant rs3890011, a variant in partial linkage disequilibrium with rs1126742, would be associated with resistant hypertension. To test this hypothesis we used the Department of Veteran Affairs Million Veterans Program dataset. The dataset is derived from electronic medical records using electronic algorithms to identify patients with resistant hypertension (cases, n=16,833), defined as uncontrolled blood pressure of at least 140/90 mmHg despite the concurrent use of three antihypertensive medications including a thiazide diuretic or patients on four or more antihypertensive medications including a thiazide diuretic, and patients with controlled blood pressure (controls, n=53,931) less than 135/90 mmHg on one or two antihypertensive medications. Within this population we found that there was a significant

association between both rs3890011 and rs1126742 and resistant hypertension (β=0.05, p<0.001; β=0.04, p=0.02, respectively). When we stratified the population by race, rs3890011 was significantly associated with resistant hypertension in both whites and blacks (β=0.04, p<0.001 and β=0.06, p=0.02, respectively) while rs1126742 was associated with resistant hypertension in blacks (β=0.07, p=0.01) but not whites. These findings support an association between these *CYP4A11* loss-of-function variants and resistant hypertension.

3.2 Introduction

Resistant hypertension (defined as elevated blood pressure (BP) greater than 140/90 mmHg despite concurrent use of three antihypertensive medications including a thiazide diuretic) increases a patient's risk for developing coronary heart disease, stroke, and chronic kidney disease.[167, 168] In some patients with pseudo-resistant hypertension the lack of BP control may be explained by inadequate treatment or medication nonadherance hypertension; in the remaining patients with true resistant hypertension the lack of BP control may result from pathophysiological processes that are not targeted by current antihypertensives. The identification of these processes may, in turn, allow for the identification of new therapeutic targets for BP treatment.

Because excess dietary salt intake is common in patients with hypertension and because increased blood pressure in response to excess sodium ingestion contributes to antihypertensive resistance we have been interested in the molecular processes underlying salt-sensitive hypertension.[5, 169] One pathway involved in the BP response to salt is the formation of the eicosanoid 20-hydroxyeicosatetraenoic acid (20-HETE) by the ω-hydroxylation of arachidonic acid by cytochrome P450 enzymes, encoded specifically by members of the CYP4A and CYP4F

gene families. Inhibitors of 20-HETE formation cause salt-sensitivity in normotensive rats, while administration of clofibrate to induce CYP4A expression in Dahl salt-sensitive/JR rats can prevent salt-sensitive increases in BP.[170-172] Further, the CYP4A2 genotypes cosegregate with salt-sensitive hypertension in the F2 cross of Dahl salt-sensitive rats and normotensive Lewis rats.[173]

Depending on the location of expression in the kidney nephron, 20-HETE can either raise or lower BP. When expressed in the vasculature, 20-HETE exerts prohypertensive effects by causing vasoconstriction of the renal microvasculature.[65] When expressed in the tubules, however, 20-HETE exerts antihypertensive effects by promoting natriuresis through inhibition of the Na/K-ATPase in the proximal tubules and thick ascending limb of the loop of Henle (TALH) and inhibition of the Na-K-2Cl cotransporter in the TALH.[69-73] In humans, the antihypertensive effects of 20-HETE appear to predominate over the pro-hypertensive effects based on genetic association studies of loss-of-function variants of *CYP4A11*.

In humans, 20-HETE is formed predominantly by CYP450 enzymes encoded by the genes *CYP4A11* and *CYP4F2*. Our group has demonstrated that a variant in *CYP4A11*, rs1126742 (c.8590C>T resulting in Phe434Ser), encoding a CYP4A11 enzyme that demonstrates a 50% decrease 20-HETE synthase activity in vitro.[79] This variant has been associated with increases in blood pressure or hypertension in African American men with hypertensive nephrosclerosis, in a European population survey, European survivors of myocardial infarction, males from a western Chinese Han population, the Framingham Offspring Trial, and a Tennessee case-control study suggesting that the effect of 20-HETE or other products of CYP4A11 in humans are anti-hypertensive.[77, 79, 81-83] The rs1126742 variant was also associated with salt-sensitive hypertension in patients from the International Hypertensive Pathotype cohort.[76] An

intronic variant, rs3890011, in partial linkage disequilibrium with rs1126742, has also been associated with hypertension.[85, 86] Further, 20-HETE excretion has been shown to be regulated by salt intake in hypertensive patients.[174] Taken together with the rodent data, these findings suggest that decreased 20-HETE formation may underlie salt-sensitive hypertension and potentially resistant hypertension.

We, therefore, hypothesized that the loss-of-function polymorphisms in *CYP4A11* would be associated with resistant hypertension. We tested this hypothesis using the Department of Veteran Affairs Million Veterans Program (MVP), a large DNA databank linked with an electronic medical record (EMR). Patients with resistant and controlled hypertension were identified using electronic algorithms. Resistant hypertensive cases were defined as patients with BP equal to or greater than 140/90 mmHg despite concurrent use of three antihypertensive medications including a thiazide diuretic or patient prescribed four or more concurrent antihypertensive medications including a thiazide regardless of BP. The controlled hypertension comparator group included patients with BP controlled to less than 135 mmHg systolic or less than 90 mmHg diastolic who were prescribed one or two antihypertensive medications. All subjects were genotyped using a customized version of the Affymetrix Axiom Biobank Array and the rs1126742 and rs3890011 genotypes were imputed to the 1000 Genomes reference panel.

3.3 Methods

Case and Control Identification from the Electronic Health Record

Using electronic algorithms, patients with resistant hypertension (cases) and patients with controlled hypertension (controls), were identified from 510,167 veterans enrolled in the VA MVP using the electronic health records (EHR).[117] These algorithms are modified from a

previously published algorithm to define resistant hypertension within the electronic Medical Records and Genomics (eMERGE) network. [105, 120] These algorithms will be validated by blinded electronic record review.

Resistant hypertensive cases were defined as patients with uncontrolled BP of at least 140 mmHg systolic or at least 90 mmHg diastolic despite concurrent use of three or more antihypertensive medications including a thiazide diuretic or patients who achieved control on four or more antihypertensive medications including a thiazide. All BPs were collected in the outpatient setting. Outpatient BPs collected on a day when a patient had an emergency department visit were excluded. Outpatient BPs collected on days with an associated pain score of five or during use of an interfering medication- tacrolimus, cyclosporine, epoetin, or darbepoetin- were also excluded. Patients with confounding medical conditions listed in **Table 9** were excluded from the final population.

Controlled hypertensive patients were selected to have outpatient BPs less than 135/90 mmHg while prescribed one or two antihypertensive medications from a larger BP cohort.

All patient characteristics- age, gender, race, body mass index (BMI), BPs, estimated glomerular filtration rate (eGFR), calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, and history of type 2 diabetes mellitus (T2DM)- were extracted from the EHR using the International Classification of Disease (ICD)-9 codes, laboratory measures and natural-language processing.[175, 176] The reported age is the age the patient was during the visit they met the criteria for inclusion as a case or control. BMI was calculated using the most frequent height reported in a patient's medical record and the closest weight collected to the inclusion date within 189 days after inclusion or 365 days prior to inclusion. Baseline eGFR was estimated based on the closest eGFR available to the subject

inclusion date. Subjects with one or more eGFR measures less than 60 ml/min/1.73m^2 collected within a year of identification were excluded due to the presence of preexisting chronic kidney disease (CKD). The reported systolic (SBP) and diastolic (DBP) blood pressures for controls were the median SBP across the EHR along with the first corresponding DBP. For cases, the reported SBP and DBP are determined from the first BP collected between 21 days after meeting medication criteria inclusion and six months after inclusion. Patient race was determined by administrative assignment.

After using the described algorithm to identify cases and controls in the total MVP population of 510,358 veterans, we restricted the population for analysis to those patients for whom genotypic and outpatient BP data were available, 318,895 veterans. We also studied only patients meeting the case/control inclusion criteria administratively assigned a race-ethnicity of non-Hispanic African American (AA) or non-Hispanic European American (EA).

CYP4A11 Genotyping

Blood samples were drawn from consenting MVP participants at local VA sites and then shipped to the VA Central Biorepository in Boston, MA where DNA was extracted and shipped to two genotyping centers. The DNA from 123,606 (24.2%) MVP veterans were genotyped using a customized version of the Affymetrix Axiom Biobank Array, with approximately 723,000 markers that is enriched for exonic SNPs, validated disease associated tag SNPs, and African American and Hispanic ancestry markers.[117] The MVP genomics working group applied standard quality control and genotype calling algorithms to the batched data using the Affyemtrix Power Tools Suites (v1.18). Duplicate samples, samples with more heterozygosity than expected under Hardy-Weinberg equilibrium, samples with missing genotype rates

exceeding 2.5%, and samples with discordance of genetically inferred sex versus self-report were excluded using standard quality control pipelines. Related individuals (halfway between second and third degree relatives or closer) as measured by the KING software were also excluded.[177]

The rs1126742 and rs3890011 variants were imputed, not genotyped directly on the array. Following standard quality control cleaning and filtering, described above, the remaining variants that were poorly called or deviated from the expected allele frequency based on reference data from the 1000 Genomes Project were excluded.[178] The remaining variants were pre-phased using Eagle2 and then imputed against the 1000 Genomes Project phase 3 version 5 reference panel using miniMac3 software. [178-180] Principle component analysis was performed using the FlashPCA, to generate the top 10 genetic principle components explaining the greatest amount of genetic variability.[181]

Statistical Analyses

Data are presented as frequencies for categorical variables and median and interquartile range for continuous variables. Pearson chi-square tests and Wilcoxon rank-sum tests were run for between-group comparisons for categorical and continuous variables, respectively. All statistical analyses were run using R 3.3.0.[133]

The genetic association analyses were conducted using linear regression association tests with additive models for case/control determination. We adjusted for age at case/control determination date, age^2, sex, BMI within a year of case/control determination, and the top 10 genetic principle components in the linear regression models for SNP association. All primary analyses were stratified by race using either administratively assigned race/ethnicity or by

empirically designated clusters. These strata were then meta-analyzed using METAL.[182] All regression-based analyses were conducted in SNPTEST v2.5.4-beta.[183]

3.4 Results

Identification of resistant hypertension cases and controlled hypertension

From a total population of 318,895 MVP veterans, 53,931 (16.9%) veterans were identified as EA or AA with controlled hypertension (controls) and 16,833 (5.3%) veterans were identified as EA or AA resistant hypertensive cases. Among controls, 42,850 (79.5%) were EA and 11,081 (20.5%) were AA. From the total resistant hypertensive population, 11,762 (69.9%) were white and 5,071 (30.1%) were black. The prevalence of resistant hypertension was significantly greater in AA than in EA ($p<0.001$).

Regardless of race, the cases had significantly higher BP, were older, predominately male, heavier, had lower eGFRs, and more likely to have T2DM than controls (**Table 10**). With the exception of potassium-sparing diuretics in AA all antihypertensive medication classes were prescribed more frequently in the resistant hypertension cases than in the controls (**Table 10**). Further, AA cases were significantly younger, had higher BPs, lower BMI, higher eGFR, and were more likely to be female than EA cases (**Table 11**). EA were more significantly more likely to be prescribed either an angiotensin converting enzyme inhibitor (ACEi) or angiotensin converting receptor blocker (ARB), beta blocker, alpha antagonist, or loop diuretic than AA (**Table 11**). While, AA were more likely to be a calcium channel blocker, vasodilator, or potassium sparing diuretic than EA (**Table 11**).

***CYP4A11* genotype and resistant hypertension**

CYP4A11 rs1126742 and rs3890011 genotypes were in Hardy-Weinberg equilibrium. The imputation r^2, or the squared correlation between the actual and the imputed allelic dosages at a genomic variant, were 1.0 for the1126742 variant and 0.94 for rs389011. In cases and controls combined, the minor allele frequency of the loss-of-function C allele of rs1126742 was 14% in EA and 31% in AA, consistent with the frequencies published previously in hypertensive populations.[79, 82] Consistent with previously reported frequencies, the frequency of the C allele of rs3890011 was 22% in EA and 57% in AA.[86, 184]

Both *CYP4A11* variants, rs1126742 and rs3890011, were significantly associated with resistant hypertension in the meta-analyzed total population (**Table 10**). The increased frequency of the *CYP4A11* variants in AA, as well as, an association between black race and resistant hypertension makes running a genetic analysis stratified by race essential. In AA, the rs1126742 loss-of-function C allele was significantly associated with resistant (β=0.07, p=0.01) (**Table 10**). The C allele of rs3890011 was significantly associated with resistant hypertension in both EA (β=0.04, p=0.01) and AA (β=0.06, p=0.45) (**Table 10**).

3.5 Discussion

Elucidation of pathways underlying the pathophysiology of resistant hypertension can allow for the identification of novel targets for the control of BP in these patients at an increased risk of cardiovascular events and chronic kidney disease. One approach to identifying these novel pathways are genetic association studies in large clinical populations of patients. Because hypertension is a complex trait, studying a more homogenous subset of patients such as those with resistant hypertension in larger clinical populations could allow for the identification of associations not detected in smaller populations with heterogeneous forms of BP. By using

electronic algorithms to identify EA and AA patients with resistant and controlled hypertension in VA MVP we were able to identify 59,931 veterans with controlled hypertension and 16,833 resistant hypertensive cases for use in a genetic association study. Consistent with prior studies, the frequency of resistant hypertension was greater in AA compared to EA and patient with resistant hypertension were significantly older, heavier, and had a higher incidence of T2DM than patients with controlled hypertension.[5, 135-137] Given prior evidence suggesting an association between *CYP4A11* variants and hypertension, we studied the impact of two loss-of-function variants of *CYP4A11* on RH, rs1126742 and rs3890011. We identified weak associations with small effect sizes between resistant hypertension and these two variants.

The eicosanoid 20-HETE has been shown to have both pro-hypertensive and anti-hypertensive effects depending on site-specific expression in the kidney vasculature or tubule, respectively. Studies in Dahl-SS rats suggest that the anti-hypertensive effects of 20-HETE predominate; studies in mice, however, suggest the opposite. Because the effects of 20-HETE on BP cannot be probed in humans using CYP4A11 inhibitors or 20-HETE analogs at present, studies in human with *CYP4A11* loss-of-function variants have become essential to understand these effects in humans. To date, genetic studies have shown the C allele of rs1126742 is associated with hypertension, higher SBP, salt-sensitive BP, and diminished a 20-HETE response to salt loading.[76, 77, 79, 81, 82, 84, 185]

In rat models of hypertension and in humans, a decrease in endogenous 20-HETE levels result in salt-sensitive BP.[76, 170, 172, 173, 186] Because excessive dietary sodium intake is associated with elevated BP,[187, 188] because high levels of dietary sodium have been associated with antihypertensive resistance,[189] and because a randomized control study of salt-restriction in patients with resistant hypertension resulted in a 22.7 and 9.1 mmHg decrease in office SBP and

DBP, respectively,[169] we hypothesized that a pathway underlying salt-sensitivity, such as those resulting in a decrease in 20-HETE, would be associated with resistant hypertension.[190-192] While our results support this hypothesis, our association with resistant hypertension was relatively weak, e.g. βs less than 10%. While the exact prevalence of salt-sensitive hypertension in patients with resistant hypertension is not known, various studies in patients with essential hypertension have estimated the prevalence of salt-sensitivity in hypertensive patients to be ~50%.[193-195] Since salt-sensitive BP is not unique to patients with resistant hypertension and many of the patients with controlled hypertension may also have salt-sensitive hypertension, the weak association with resistant hypertension is not surprising.[196]

We detected an association between the loss-of-function C allele of rs1126742 and resistant hypertension in the total population and in AA but not EA. The lack of association in EA may be due to insufficient power to detect such a small effect in EA. Our ability to detect the effect in AA may be due to an increase in the frequency of the C allele in AA compared to EA (31% versus 14%, respectively). We may have also detected the effect in AA but not EA because salt-sensitivity and resistant hypertension is more common in AA.[5, 190-192] In the African American Study of Kidney Disease (AASK) trial, *CYP4A11* rs1126742 C allele carriers tended to be prescribed a diuretic prior to randomization more frequently than T allele carriers, suggesting AA with hypertensive renal disease carrying the C allele may suggest these patients are more likely to have salt-sensitive hypertension. [77]

There are limitations to this study. First, the identified resistant hypertensive cases do not represent a true resistant hypertensive population but rather an apparent resistant hypertensive population because BPs were not collected using ambulatory BP monitors and there is no direct measure of medication adherence. [12, 197] Therefore, white-coat hypertension and medication

adherence could not be ruled out as potential confounders. Another limitation of this study is the use of control subjects that required more than one antihypertensive to achieve adequate BP control. Without long-term follow-up of the identified controls to rule out more advanced hypertension later in life, including the possibility that a subset of the control subjects may eventually become cases, we cannot assure a completely homogenous control population. Finally, our *CYP4A11* variants were not directly genotyped using the Affymetrix Array platform and the imputation results were not independently validated. Because the imputation r^2 values were high, however, e.g. rs1126742 r^2=1.0 and rs3890011 r^2=0.94 meaning the software has 100% and 94% confidence in the predicted dosages respectively, inaccuracy in the imputed genotype calls is not of great concern.

In conclusion, our work supports a relationship between *CYP4A11* loss-of-function variants and resistant hypertension. It also suggests that future work to better understand the role of salt-sensitive BPs in patients with resistant hypertension and to evaluate the relationship between 20-HETE and salt-sensitivity in patients with resistant hypertension is warranted.

3.6 Conclusions

In conclusion, electronic algorithms to identify patients with resistant hypertension and nonresistant hypertension in the VA's MVP can be used to evaluate novel pathways using genetic association studies. We found a weak association between two variants in *CYP4A11* that result in a decrease in the formation of the eicosanoid 20-HETE and resistant hypertension. Because lower levels of endogenous 20-HETE have been associated with salt-sensitive hypertension, because blacks are more likely than whites to have salt sensitive BP, because the frequency of resistant hypertension is greater in blacks, and because the loss-of-function C allele

of rs1126742 was associated with resistant hypertension in blacks but not whites our data suggests that the detected association may actually reflect differences in salt-sensitivity.

3.7 Tables

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Table 9. ICD-9, ICD-10, and CPT codes excluded by the algorithm definitions

Description	ICD-9-CM codes	ICD-10-CM codes	CPT codes
Exclusion for Case Type I, Case Type II, and Control if ever present in a patient record			
Dialysis (Requires two outpatient codes 90 days apart or an inpatient code and an outpatient code 90 days apart)	V45.11, V56.0 39.93, 39.95, 54.98	Z49.31, Z99.2 031A0JF, 031B0JF, 031C0JF, 03130JD,03140JD, 03150JD, 03170JD,03160JD,03180JD, 03190JF	90920, 90921, 90924, 90925, 90935, 90937, 90945, 90947, 90960, 90961, 90962, 90966, 90970, 90989, 90993, 90999, 99512
Transplant	996.81, V42.0, V42.1, V42.6, V42.7, 33.5*, 50.51, 50.59, 50.61, 55.69		32851, 32852, 32853, 32854, 33935, 33945, 47135, 47136, 50360, 50365, 50380
Exclusion for Case Type I and Case Type II if present in a patient record 5 years before or 1 year after identification as a Case			
Cortico-adrenal disorders	227, 255.3, 255.6		
Thyroid disorders	240, 240.9, 241, 241.1, 241.9, 242, 242.01, 242.1, 242.11, 242.2, 242.21, 242.3, 242.31, 242.4, 242.41, 242.8, 242.81, 242.9, 242.91, 243, 244*, 245*, 246*		
Parathyroid disorder	252, 252.01, 252.02, 252.08, 252.1, 252.8, 252.9		
Sleep Apnea	327.2*		
Secondary Hypertension	405.01, 405.09, 405.11, 405.19, 405.91, 405.99		
Obstructive uropathy	599.6*		

Abbreviations: ICD-#-CM, International Classification of Disease, revision number, Clinical Modification; CPT, Current Procedural Terminology

Table 10. Characteristics of nonresistant hypertensive controls and resistant hypertensive cases stratified by race at diagnosis.

Variable	European Americans			African Americans		
	Controls	**Cases**	**p-value**	**Controls**	**Cases**	**p-value**
	N=42,850	N=11,762		N= 11,081	N= 5,071	
SBP	129 (123.0-134.0)	144 (132.0-154.0)	<0.001	130 (124.0-134.0)	145 (133.0-155.0)	<0.001
DBP	76 (70.0-81.0)	79 (71.0-88.0)	<0.001	79 (72.0-84.0)	85 (76.0-93.0)	<0.001
Age	62 (56.0-67.0)	63 (58.0-68.0)	<0.001	56 (50.0-62.0)	58 (53.0-64.0)	<0.001
BMI	29.7 (26.5-33.5)	31.71 (28.2-35.9)	<0.001	29.56 (26.3-33.5)	31.26 (27.5- 35.4)	<0.001
Female, n (%)	1,873 (4.3%)	318 (2.7%)	<0.001	1,181 (10.6%)	296 (5.8%)	<0.001
Baseline eGFR	82.10 (72.0-93.2)	81.18 (71.0-92.2)	<0.001	89.39 (77.0 -104.0)	85.76 (73.9- 100.4)	<0.001
T2DM, n (%)	13,048 (30.4%)	4,888 (41.5%)	<0.001	3,799 (34.2%)	2,148 (42.3%)	<0.001
Antihypertensive Medications						
ACEi/ARB	21453 (50.0%)	10487 (89.2%)	<0.001	5159 (46.6%)	4429 (87.3%)	<0.001
Beta Blockers	14167 (33.1%)	7945 (67.5%)	<0.001	2009 (18.1%)	3006 (59.3%)	<0.001
CCBs	5811 (13.6%)	6395 (54.4%)	<0.001	2546 (23.0%)	3420 (67.4%)	<0.001
Alpha blockers	8500 (19.8%)	3823 (32.5%)	<0.001	1720 (15.5%)	1414 (27.9%)	<0.001
Vasodilators	23 (0.05%)	247 (2.1%)	<0.001	17 (0.15%)	218 (4.3%)	<0.001
Thiazide diuretics	8075 (18.8%)	11762 (100%)	<0.001	3601 (32.5%)	5071 (100%)	<0.001
Loop diuretics	1063 (2.5%)	1007 (8.6%)	<0.001	209 (1.9%)	380 (7.5%)	<0.001
K^+sparing diuretics	1359 (3.2%)	510 (4.3%)	<0.001	541 (4.9%)	274 (5.4%)	0.17
CYP4A11 genotype						
rs1126742			0.45			0.007
TT	31,794 (74.2%)	8,692.5 (73.9%)		5,033 (47.8%)	2,329.5 (46.0%)	
TC	10,227.7 (23.9%)	2,830.7 (24.1%)		4,508.7 (42.8%)	2,203.9 (43.5%)	
CC	828.4 (1.9%)	238.8 (2.0%)		984.3 (9.4%)	534.7 (10.6%)	
rs3890011			0.02			0.02
GG	25,973.2 (60.6%)	7,012.4 (59.6%)		1,949.4 (18.5%)	1,949.4 (18.5%)	

GC	14,776.8 (34.5%)	4,141.9 (35.2%)	5142.3 (48.9%)	2,403 (47.4%)
CC	2,097.6 (4.9%)	607 (5.2%)	3,434 (32.6%)	1,762.9 (34.8%)

Abbreviations- ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; BMI, body mass index; CCBs, calcium channel blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

Data are presented as median (interquartile range) unless otherwise indicated.

Table 11. Characteristics of European American and African American patients with controlled hypertension (controls) and resistant hypertension (cases)

	Controls			Cases		
Variable	**EA**	**AA**	**p-value**	**EA**	**AA**	**p-value**
	N=42,850	N= 11,081		N=11,762	N= 5,071	
SBP	129 (123.0-134.0)	130 (124.0-134.0)	<0.001	144 (132.0-154.0)	145 (133.0-155.0)	<0.001
DBP	76 (70.0-81.0)	79 (72.0-84.0)	<0.001	79 (71.0-88.0)	85 (76.0-93.0)	<0.001
Age	62 (56.0-67.0)	56 (50.0-62.0)	<0.001	63 (58.0-68.0)	58 (53.0-64.0)	<0.001
BMI	29.7 (26.5-33.5)	29.56 (26.3-33.5)	0.001	31.71 (28.2-35.9)	31.26 (27.5- 35.4)	<0.001
Female, n (%)	1,873 (4.3%)	1,181 (10.6%)	<0.001	318 (2.7%)	296 (5.8%)	<0.001
Baseline eGFR	82.10 (72.0-93.2)	89.39 (77.0-104.0)	<0.001	81.18 (71.0-92.2)	85.76 (73.9- 100.4)	<0.001
T2DM, n (%)	13,048 (30.4%)	3,799 (34.2%)	<0.001	4,888 (41.5%)	2,148 (42.3%)	0.30
Antihypertensive Medications						
ACEi/ARB	21453 (50.0%)	5159 (46.6%)	<0.001	10487 (89.2%)	4429 (87.3%)	<0.001
Beta Blockers	14167 (33.1%)	2009 (18.1%)	<0.001	7945 (67.5%)	3006 (59.3%)	<0.001
CCBs	5811 (13.6%)	2546 (23.0%)	<0.001	6395 (54.4%)	3420 (67.4%)	<0.001
Alpha blockers	8500 (19.8%)	1720 (15.5%)	<0.001	3823 (32.5%)	1414 (27.9%)	<0.001
Vasodilators	23 (0.05%)	17 (0.15%)	0.001	247 (2.1%)	218 (4.3%)	<0.001
Thiazide diuretics	8075 (18.8%)	3601 (32.5%)	<0.001	11762 (100%)	5071 (100%)	1.0
Loop diuretics	1063 (2.5%)	209 (1.9%)	<0.001	1007 (8.6%)	380 (7.5%)	<0.001
K^+sparing diuretics	1359 (3.2%)	541 (4.9%)	<0.001	510 (4.3%)	274 (5.4%)	0.003

Abbreviations- ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; BMI, body mass index; CCBs, calcium channel blockers; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

Data are presented as median (interquartile range) unless otherwise indicated.

CHAPTER 4

DETERMING THE BLOOD PRESSURE RESPONSE TO SPIRONOLACTONE IN PATIENTS WITH RESISTANT HYPERTENSION USING ELECTRONIC MEDICAL RECORDS

4.1 Abstract

Spironolactone is recommended for the treatment of resistant hypertension. Using the Vanderbilt University Medical Center DNA repository, BioVU, we identified European American and African American patients with resistant hypertension who were prescribed spironolactone during a period of otherwise stable medication use and calculated the blood pressure response. The mean decrease in systolic blood pressure following spironolactone was 8.5±18.1 mmHg and the mean decrease in diastolic blood pressure was 4.0±9.9 mmHg, consistent with data from published clinical trials. Using a mean decrease in systolic blood pressure of 5 mmHg or in diastolic blood pressure of 2 mmHg to distinguish "responders" from "nonresponders", we determined that 29% of patients prescribed spironolactone did not respond. Responders had significantly higher baseline blood pressures, were older, and had larger decreases in the estimated glomerular filtration rate and serum sodium and increases in creatinine and serum potassium after starting spironolactone than nonresponders. In African Americans, glucose increased following spironolactone in responders compared to non-responders (2.27±40.77 vs -15.47±62.94, p=0.03); this relationship was not observed in European Americans. When response was evaluated as a continuous variable, the decrease in systolic and diastolic blood pressures correlated with the decrease in serum sodium and

with the increases in serum potassium and creatinine. In African Americans there was a significant correlation between decreases in systolic blood pressure and increases in glucose (p=0.04). In conclusion, we have developed an algorithm to assess the blood pressure response to a commonly prescribed medication, spironolactone, in patients with resistant hypertension using electronic medical records. Electrolyte changes associated with the blood pressure response to spironolactone are consistent with its mechanism of action to block the mineralocorticoid receptor and decrease activity of the epithelial sodium channel.

4.2 Introduction

The mineralocorticoid receptor (MR) antagonist spironolactone has been identified as the most effective add-on therapy for blood pressure (BP) control in patients with true resistant hypertension in studies including the Prevention and Treatment of Hypertension with Algorithm based therapy-2 (PATHWAY-2) trial and the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA).[51, 198-200] In the PATHWAY-2 trial, the addition of spironolactone at a dose of 25-50 mg/day to a therapeutic regimen containing an angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB), calcium channel blocker (CCB), and thiazide-like diuretic significantly decreased home systolic blood pressure (SBP) by a mean of 8.7 mmHg.[51] In prior clinical studies the SBP/diastolic blood pressure (DBP) responses to spironolactone have ranged from a mean decrease of 4.6/1.8 mmHg to a mean decrease of 25/12 mmHg.[51, 198-201]

We hypothesized that we could use the electronic medical records (EMR) to assess the BP response to the addition of spironolactone in resistant hypertensive patients who were on a stable anti-hypertensive regimen of at least three medications including a thiazide diuretic or CCB. We used a previously published algorithm for the identification of patients with resistant hypertension. To evaluate the BP response to spironolactone we developed an algorithm to identify patients who were prescribed spironolactone during a period of stable medication use from up to six months before the start of spironolactone to six months after. During the period of stable medication use the accuracy of the algorithm was validated by chart review. We collected all outpatient BPs and laboratory measures before and after spironolactone during this period.

We assessed BP response as a continuous variable and as a dichotomized variable (responders versus non-responders) and built a predictive model that included electrolyte measures relevant to the mechanism of action of MR antagonism with spironolactone.

4.3 Methods

Electronic Medical Record

We obtained Institutional Review Board approval to access the Vanderbilt University Medical Center (VUMC) DNA repository BioVU. BioVU is comprised of DNA samples extracted from discarded clinical blood samples linked to a de-identified derivative of longitudinal EMR for which Health Insurance Portability and Accountability Act of 1996 (HIPAA) identifiers are removed by established de-identification software as well as custom techniques.[119]

Spironolactone Response Algorithm Development

Patient records in BioVU were identified as having resistant hypertension by using the two possible case definition algorithms outlined in **Chapter 2**.[202] Patients were defined as having resistant hypertension if their BP remained uncontrolled, with a BP greater than or equal to 140/90 mmHg, despite concurrent use of three antihypertensives including a thiazide diuretic or dihydropyridine (DHP) CCB, or if they were taking four or more antihypertensive medications, including a thiazide diuretic or DHP CCB. Patients with secondary hypertension, chronic kidney disease (CKD) stages four and five, heart failure with reduced ejection fraction less than 35, thyroid and parathyroid disorders, nephrotic syndrome, chronic glomerulonephritis, anomalies of the bulbus cordis, coarctation of the aorta, adrenal gland neoplasms and disorders (excluding adrenal insufficiencies), chronic pulmonary heart disease, thyrotoxicosis, disorders of thyrocalcitonin secretion, and obstructive uropathy were excluded by the resistant hypertension algorithm.

All drug exposures to antihypertensive medications including spironolactone were identified from BioVU by electronic-prescribing tools and MedEx,[125] and at least one of the following identifiers, dose, route, frequency, or duration, were required to consider a medication exposure valid. The utility of these tools for extracting medication data from the EMRs has been shown previously.[126, 127]

We then developed an algorithm to identify patients in BioVU who were prescribed spironolactone and for whom BP was measured within a stable window of time before and after initiation of spironolactone. Using electronic prescribing tools and MedEx,[125] the novel start date (NSD) for a spironolactone prescription was determined

by the earliest mention of spironolactone, Aldactone, or Aldactazide in a patient's record after meeting the resistant hypertension case definition. Further, spironolactone, Aldactone, or Aldactazide prescription has to have been listed at least twice, at least one month apart, during the subsequent six-month period. Patients who were initiated on Aldactazide were required to have been using a thiazide diuretic immediately prior to the NSD for Aldactazide. Subjects who were not prescribed a thiazide prior to the start of Aldactazide were excluded as having been started on spironolactone and hydrochlorothiazide (HCTZ) concurrently. The MR antagonist eplerenone was not included in the population as it was prescribed to only 2% of the resistant hypertensive population.

Patients prescribed spironolactone without an outpatient BP measurement in the six months before or after the NSD were excluded. Patients who were diagnosed with heart failure, hepatic cirrhosis, hyperandrogenism, acne, or polycystic ovarian syndrome within a year of the NSD were also excluded. Next, the algorithm identified sliding time windows up to six months before (baseline) and six months after the NSD (response) when each patient had no change in his or her prescribed medications, including changes to medication class, count, or dose (**Figure 9**).

Once the baseline and response periods were defined, all outpatient BP measurements taken during these periods were identified. Any patient without at least one BP measurement during the baseline period and at least two BPs measurements during the response period were excluded. For both SBP and diastolic (DBP), response was calculated as the difference between the mean BP in the stable post-treatment window minus the mean BP in the pre-treatment window (e.g. $\Delta SBP = \overline{SBPpost} -$

$\overline{SBPpre}$). We also defined patients as responders versus non-responders based on a review of BP responses reported in clinical trials of spironolactone in EA and AA.[51, 198-201] We defined responders as those who had a decrease in mean SBP of at least five mmHg or a decrease in mean DBP of at least two mmHg, corresponding to the smallest SBP and DBP responses to spironolactone reported among the studies reviewed.

All patient characteristics, age, gender, race, body mass index (BMI), outpatient BP measurements, serum potassium, creatinine, and sodium, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glucose, hemoglobin A1C, and history of chronic kidney disease (CKD) stage three, ischemic heart disease (IHD), type two diabetes mellitus (T2DM), and smoking, were extracted from BioVU using a combination of International Classification of Disease (ICD)-9 and -10 codes, Current Procedural Terminology codes, laboratory measures, and natural-language processing (**Table 12**). Aldosterone, renin, renin activity, and aldosterone-renin-ratio (ARR) were also extracted for patients during the baseline and response periods, however, these values were excluded from our analyses because zero, zero, two, and nine patients, respectively, had measures during both periods. For each patient, age and BMI at NSD or the date closest to NSD was used. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula.[128] History of CKD stage three was defined as an estimated glomerular filtration rate >30 mL/min/1.73m^2 and <60 mL/min/1.73m^2 or equivalent ICD-9 or -10 code at any point before NSD. Patient race was administratively assigned in BioVU based on either physician or patient report. Previous work has shown that self-identified race is highly correlated with genetic ancestry[129, 130] and administratively assigned race in VUMC's SD

or BioVU is sufficient for genetic association analyses[131] and correlates tightly with genetic ancestry.[129]

After the algorithms were iteratively refined, blinded chart reviews of randomly chosen, never overlapping, charts were performed to determine algorithm efficacy. Based on a population size of 977, review of 122 charts would allow estimation of a misclassification rate of 10% with a margin of error of 5%. We therefore chose to review 150 charts to determine algorithm efficacy. The review consisted of 75 charts from resistant hypertensive patients that were included by the algorithm and 75 that were excluded. The algorithm was refined until a negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity greater than 90% was achieved based on the review of 150 charts. The final version of the algorithm will be available at Phenotype KnowledgeBase (PheKB).[203]

Statistical Methods

Data are presented as frequencies for categorical variables and mean and standard deviations for continuous variables. Between-group comparisons were made using Pearson's chi-squared test for categorical variables and the Wilcoxon test for continuous variables. All continuous variable correlations were run using a Pearson test. Multivariable logistic regression models for BP response were fit for race (in the total population), age, gender, body mass index (BMI), history of T2DM, diuretic and CCB use, spironolactone dose, and baseline SBP and DBP. All statistical analyses were run using the SPSS software version 24 (SPSS, Chicago, IL, USA) or R 3.3.0.[133]

4.4 Results

Resistant hypertension in BioVU

Using the resistant hypertension algorithms developed in **Chapter 2** we identified 17,082 EA and AA subjects from the VUMC SD with resistant hypertension. Case type one patients were patients simultaneously prescribed three antihypertensive medications including a thiazide diuretic or DHP CCB with a six month mean BP greater than 140/90 mmHg. Case type two patients were patients prescribed four or more antihypertensive medications including a thiazide diuretic or DHP CCB. If both algorithms identified the same patient the patient was removed from the case type one population and remained part of the case type two population.

We determined that 6,695 EA (49.4%) and 1,665 (47.0%) AA subjects with resistant hypertension had DNA collected in BioVU. The EA and AA subjects from BioVU were significantly more likely to have T2DM and sleep apnea at initial diagnosis than those in the total SD (**Tables 13 and 14**). The EA subjects from BioVU were also older, less frequently prescribed BB, and more frequently prescribed thiazide diuretics at initial diagnosis than the total SD population **(Table 13**).

Algorithm validation

NPV, PPV, sensitivity, and specificity of the algorithm for spironolactone response were determined after blinded review of 150 electronic records. All excluded records were excluded appropriately. Six of 75 records included in the algorithm should have been excluded. In one patient included, the spironolactone mention did not have an accurate dose associated with its use at any point therefore the subject should have been

excluded. Three subjects were called as cases, however, during the baseline or response periods they had an inconsistent medication mention, e.g. for one or more visits there was a different medication listed then those listed for the other visits. For two of these patients these medication mentions were single occurrences and are not a true change in the medication record, however, for the other patient the medications are mentioned more than once making it difficult to confirm the actual prescription. One patient had spironolactone listed in the record multiple times as a part of a note to consider addition with no indication the medication was actually prescribed. One patient did not have a second BP measurement at least a month after the first BP in the six months following the start of spironolactone. The NPV and sensitivity were 100%, specificity 93%, and PPV 92%.

Identification of spironolactone response population

Among EA and AA patients with resistant hypertension in BioVU 1,651 EA and 455 AA were ever prescribed spironolactone. After applying exclusion and inclusion criteria, 751 EA patients and 226 AA patients were included in the study for evaluation of spironolactone response, 45.5% and 49.7% of the original number of patients prescribed spironolactone, respectively (**Figure 10**). The median dose of spironolactone prescribed was 25 mg and the dose ranged from 5 to 100 mg. The majority of patients included in the study, 724 patients, were prescribed 25 mg (74.1%).

Characteristics of spironolactone responders and nonresponders

Defining spironolactone response as a reduction in SBP of five mmHg or in DBP of two mmHg, we identified 694 responders and 283 non-responders (29%). Of the responders, 125 patients met the criteria for a DBP response, 118 for a SBP response, and 451 patients met criteria for both DBP and SBP response. Patients who responded to spironolactone had a significantly higher baseline SBP ($p<0.001$), DBP ($p<0.001$), and serum sodium ($p=0.003$) than patients who did not (**Table 15**). Responders were older and had a significantly greater decrease in eGFR and serum sodium and greater increase in serum potassium and creatinine than nonresponders (**Table 15**). In a multivariable model, all associations persisted except for baseline sodium.

Among AA patients 57 (25.2%) did not respond to spironolactone. The AA patients who responded to spironolactone were significantly older ($p=0.03$), and had higher baseline SBP ($p<0.001$) and DBP ($p=0.001$) than nonresponders (**Table 16**). Further, serum sodium decreased in responders but increased in nonresponders, while glucose and hemoglobin A1c (HbA1c) increased in responders, but decreased in nonresponders (**Table 16**). In multivariable analysis all of the associations persisted except the association with HbA1c ($p=0.14$).

Among EA patients, 226 (30.1%) did not respond to spironolactone. Like AA responders, EA responders had higher baseline SBP (<0.0001) and DBP (<0.001) (**Table 17**). EA responders also had significantly higher baseline serum sodium levels ($p=0.02$) and a greater increase increases in serum potassium ($p<0.001$) and creatinine ($p=0.01$) than nonresponders as well as a significantly greater decrease in eGFR ($p<0.001$) and serum sodium ($p=0.05$) (**Table 17**). In multivariable analysis all of the associations

remained statistically significant except those with baseline sodium and the decrease in sodium (p=0.238 and p=0.167, respectively).

Blood pressure response to spironolactone as a continuous variable

In the overall population the mean SBP decreased 8.5 $\pm$ 18.1 mmHg and mean DBP decreased 4.0 $\pm$ 9.9 mmHg following spironolactone treatment. There was a significant linear correlation between the decrease in SBP and decrease in DBP, baseline serum potassium, decrease in serum sodium, increase in serum potassium, and increase in creatinine. The decrease in DBP correlated significantly with baseline potassium, decrease in serum sodium, increase in serum potassium, increase in creatinine, and higher baseline sodium (**Table 18**).

In AA there was a significant positive relationship between the decrease in SBP and decrease in DBP, decrease in serum sodium, increase in creatinine, and increase in glucose. The decrease in DBP correlated significantly with the decrease in serum sodium and increases in serum potassium and in creatinine (**Table 18**).

In EA, the decrease in SBP correlated with the decrease in DBP, baseline creatinine, and baseline serum potassium, and increases in creatinine and serum potassium. The decrease in DBP correlated significantly with baseline HDL and potassium, higher baseline sodium, and increases in creatinine and serum potassium (**Table 18**).

Results of the multivariable analysis appear in **Table 18**.

4.5 Discussion

We have developed a highly accurate algorithm to define the BP response to spironolactone in patients with resistant hypertension from EMRs. Among all patients with resistant essential hypertension prescribed spironolactone for the treatment of hypertension, approximately half of the patients had sufficient BP in the six months prior to and following the start of spironolactone to evaluate response.

The mean decreases in SBP and DBP for the total population, 8.5 mmHg and 4.0 mmHg, respectively, are consistent with responses reported previously, including those reported by the Prevention and Treatment of Hypertension with Algorithm based therapy-2 (PATHWAY-2) trial.[51] In total, 283 of the patients prescribed spironolactone (29%) did not achieve a five mmHg decrease in SBP or two mmHg decrease in DBP in the six months following initiation of spironolactone. There was a slightly greater percentage of non-responders among EA than AA patients (30.1% vs. 25.2%). This difference in response may reflect a higher baseline BP in AA than EA patients as the magnitude of BP response was significantly associated with a higher BP regardless of race and there was no difference in distribution of racial groups.

Previous work has shown 24-hour urine sodium to be an independent predictor of SBP response to spironolactone, however, these values were unavailable in our population.[204] Because 24-hour urine sodium is a measure of sodium intake and increases or decreases in dietary salt intake can change plasma sodium and because changes in serum sodium can alter extracellular volume which may in turn increase or decrease BP we chose to test for an association between BP response and serum sodium.[205-207] The relationship between serum potassium and BP response to spironolactone varies in prior studies.[52, 198, 204, 208] In our study, we found significant

associations between decrease in BP and higher serum sodium and lower serum potassium at baseline, but these associations did not persist after adjustment for covariates.

We also found that decreasing SBP correlated with lower baseline creatinine in the total population and EA. While all populations showed a significant correlation between decreasing BP and increasing creatinine after starting spironolactone. An increase in creatinine can result from hemodynamic effects of blocking the renin-angiotensin-aldosterone system (RAAS). Clinical trials of ACEis and ARBs also demonstrate an initial decrease eGFR or increase in serum creatinine with RAAS blockade even as these drugs slow the rate of renal decline.[209, 210] This transient increase in eGFR was not associated with long-term harm. The National Kidney Foundations-Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines, however, suggest ACEi and ARB dose decrease and a more frequent monitoring of eGFR in patients with a serum creatinine increase greater than 30% after inhibition.[211] Because our findings suggest MR antagonism produces similar increases in serum creatinine as observed following RAAS inhibition by ACEi or ARBs, these guidelines in patients treated with spironolactone should be reconsidered.

By blocking the MR, spironolactone inhibits the aldosterone-stimulated increase in serum and glucocorticoid receptor kinase (SGK1) expression. This results in a decrease in the phosphorylation of Neural precursor cell-expressed developmentally downregulated (gene 4) protein (NEDD4-2) resulting in NEDD4-2 dependent downregulation of ENaC. We found that responders to spironolactone had a greater increase in serum sodium and decrease in serum potassium after MR antagonism and that

these levels correlated significantly with decrease in BP, consistent with a decrease in ENaC activity. These results suggest that nonresponders may have increased ENaC activity compared to responders.

In the present study, we also report a significant correlation between decreasing BP response and increasing glucose levels in AA with resistant hypertension. After adjusting for confounders only the relationship with glucose remained statistically significant. These results are consistent with previous clinical studies that reported increases in glucose and HbA1c after the start of spironolactone in patients with T2DM and heart failure.[199, 212] Further, spironolactone use has been associated with an increase in the incidence of T2DM in two different clinical studies.[213, 214]

The increase in glucose after spironolactone treatment in AA may be explained by an increase in endogenous aldosterone following MR antagonism. In murine models, aldosterone decreases insulin secretion through an MR-independent mechanism.[215] Further, in SHR/NDmcr-cp(cp/cp) rats that spontaneously develop symptoms of metabolic syndrome, treatment with spironolactone increases glucose and impairs glucose tolerance without impacting serum insulin levels.[216-218] Because AA have increased insulin resistance compared to EA and because excess aldosterone may contribute to the pathophysiology of both resistant hypertension and metabolic syndrome it is possible that increases in endogenous aldosterone due to MR antagonism may ultimately lead to impaired β-cell function and hyperglycemia in AA patients with resistant hypertension treated with spironolactone.[219-222] This relationship warrants greater scrutiny as long term treatment with spironolactone in this population may result in an unfavorable trade-off between BP control and insulin resistance.

Spironolactone is not the only MR antagonist available to lower BP in patients with resistant hypertension. Eplerenone has been shown to have comparable BP lowering effects to spironolactone in patients with hypertension, however, eplerenone treatment requires higher doses than used with spironolactone due to its lower potency.[223-225] In comparative studies of spironolactone and eplerenone use, eplerenone has not been shown to increase glucose or HbA1c levels.[212, 217] Spironolactone has been shown to increase endogenous aldosterone levels while eplerenone did not. [217] The chemical and pharmacokinetic properties of spironolactone compared to eplerenone may explain the discordant glucose outcomes. Spironolactone is a non-selective MR antagonist prodrug that forms multiple active metabolites including the principle metabolite, canrenone.[226] Concomitant administration of spironolactone and hydrochlorothiazide, a required medication in the identification of our resistant hypertension population, has been suggested to increase the bioavailability of canrenone.[226] Further, the highest tissue concentrations of canrenone compared to plasma levels was observed in the liver, intestines, adrenals and kidney.[226] Eplerenone, on the other hand, is a selective antagonist with a shorter half-life, a 20-fold lower *in vitro* affinity for the MR, and no progesterone or anti-androgen effect, and therefore a more limited side effect profile compared to spironolactone. [227, 228] We did not include patients with patients on eplerenone in this study because the drug was used infrequently in our resistant hypertension population.

The lack of BP response to spironolactone could result from non-adherence and a limitation of this study and many other studies is the inability to measure adherence directly in the patients prescribed spironolactone. Nonadherence alone does not likely

explain the lack of BP response in all nonresponders, however. First, it stands to reason that if a patient is not adherent to spironolactone, he or she is not adherent to other medications. Nonadherent patients, therefore, would be expected to have higher baseline BPs than adherent patients. To the contrary, we found that nonresponders had lower baseline SBP and DBP than responders and baseline SBP and DBP were significant predictors of BP response, i.e. patients with higher baseline BPs had the greatest decrease in BP. Second, nonresponders also had an increase in their serum potassium and decrease in serum sodium after starting spironolactone. If these subjects were not taking spironolactone we would not expect to observe a change in these levels. Taken together these finding suggest that nonadherence is not the predominant driver of the lack of BP response in non-responders.

Another limitation of our study was the inability to assess baseline or response levels of RAAS activity using measured renin and aldosterone levels due to limited availability of these measures. These levels could be used in the prediction model for BP response or to indicate the efficacy of MR antagonism. Previous studies, however, have reported limited value of the addition of aldosterone levels or the ARR in prediction models of BP response to MR antagonism in resistant hypertension.[229, 230]

4.6 Conclusions

To our knowledge this is the first study to estimate the prevalence of nonresponders to spironolactone in a real-world clinical population. Our results suggest physiologic differences may exist in responders and nonresponders and that genetic association studies within this population may help to identify these differences. We also determined

that AA patients with resistant hypertension who respond to spironolactone might have increases in glucose that need to be monitored following prescription.

4.7 Figures

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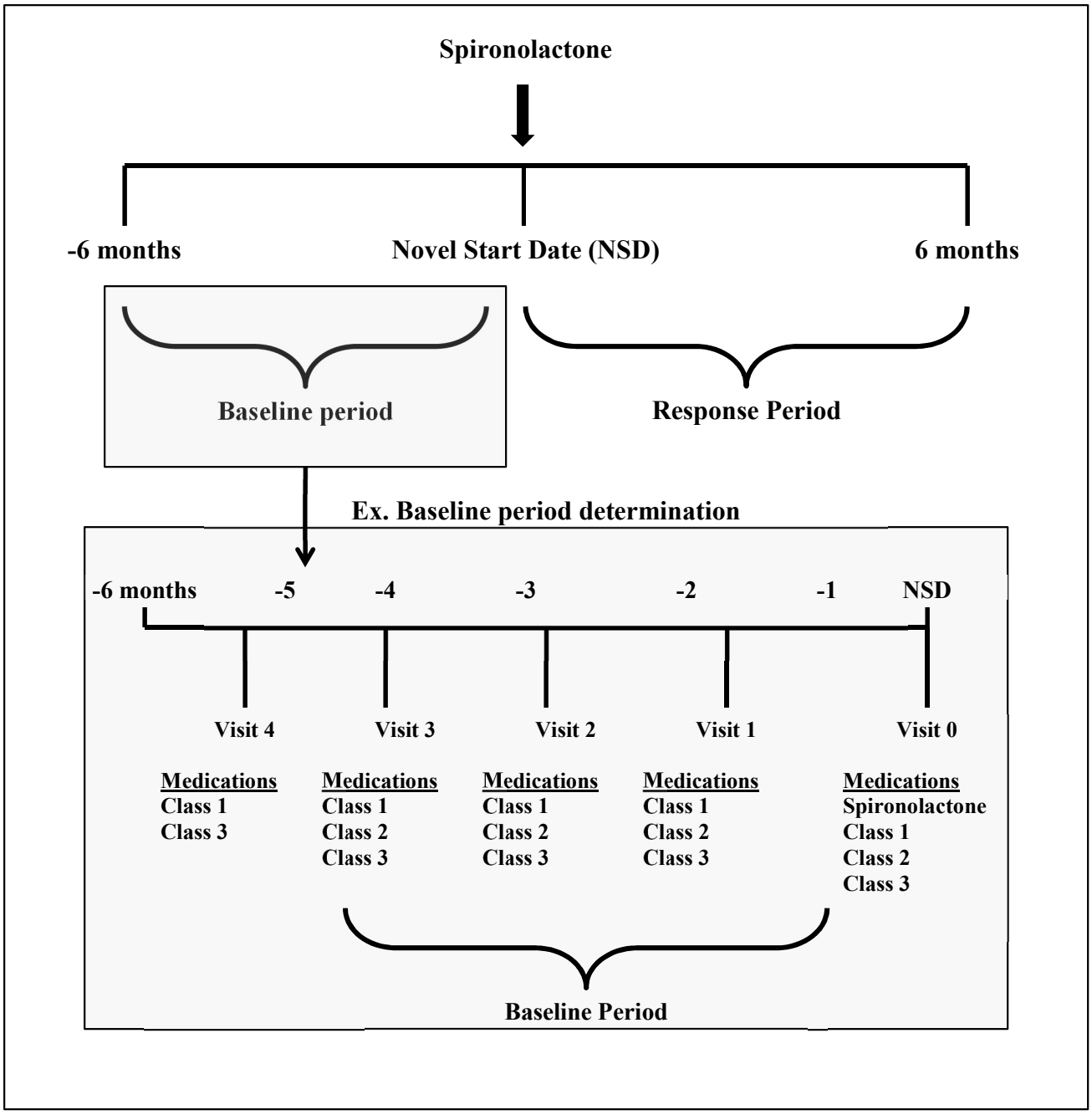

Figure 9. Schematic of the spironolactone response algorithm and identification of the baseline and response periods for patients. The earliest date a patient was prescribed spironolactone is indicated by the novel start date (NSD). The schematic then shows how the baseline period is determined. Moving back in time from the NSD the baseline period is determined by identifying all visits that have the same three medication classes as prescribed to the patient on the NSD. The visit

closest to the NSD where the medication classes are different from those prescribed on the NSD is not part of the baseline period and the visit immediately preceding this visit, Visit 4 in the example above, is determined to be the start of the baseline period. If visit date for the start of the baseline period occurs prior to six months before NSD the baseline period begins at the six-month mark. The determination of the response period is not shown in the schematic above. Similar logic is applied to this period as was used in the determination of the other with the end of the response period coming with the visit immediately preceding a visit where an antihypertensive medication class is either added or removed and if this date occurs beyond six months after NSD the period ends at the six-month mark.

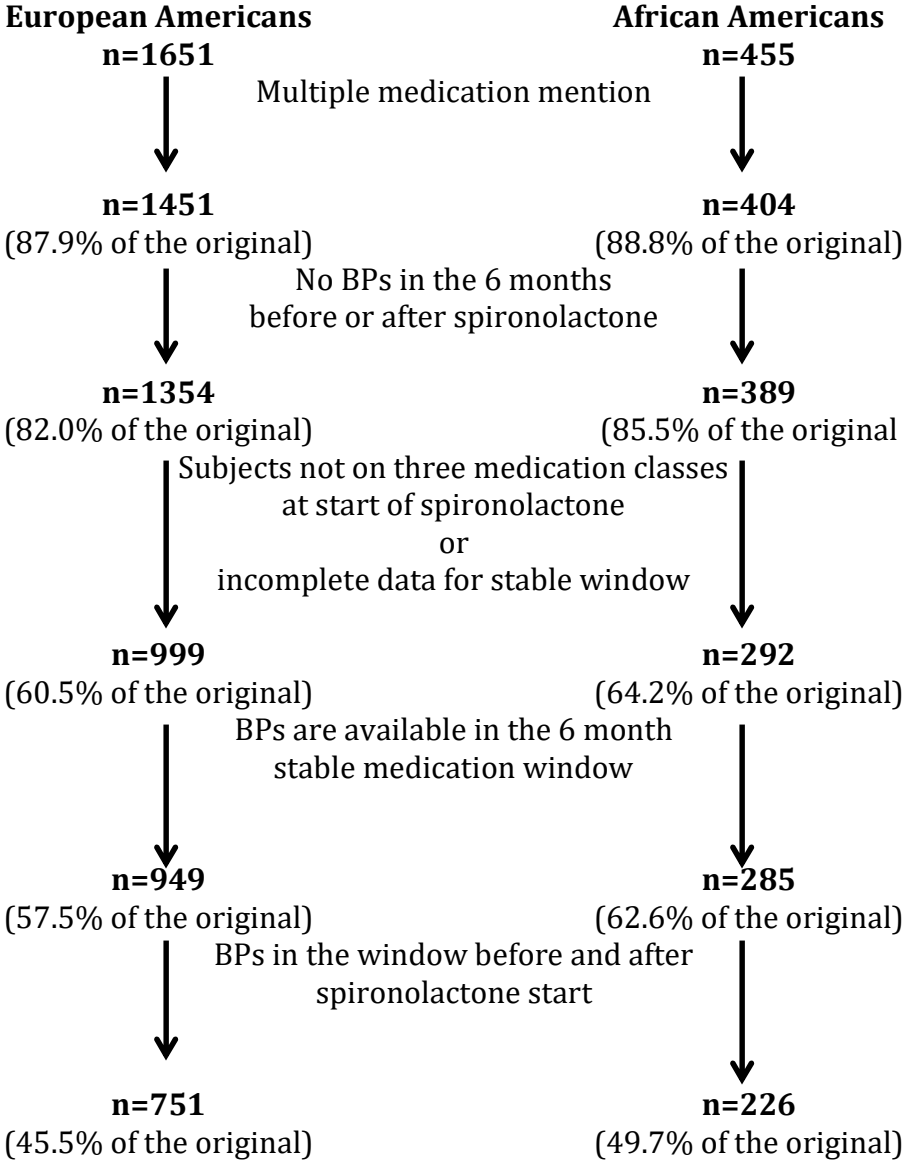

Figure 10. Diagram of the algorithm for the identification of patients with resistant hypertension in BioVU prescribed spironolactone with stable medication windows for evaluation of blood pressure response. From the total population of resistant hypertensive patients prescribed spironolactone (1,651 European Americans and 455 African Americans) we first excluded subjects that did not have spironolactone listed in his or her record more than once at least a month apart. At this point we also excluded all

subjects who had a spironolactone start date within a year of an excluded ICD-9 or ICD-10 codes. Subjects were then excluded if they did not have blood pressure measurements in the six months before or after spironolactone start; if they were not on three antihypertensive medications on the day they started spironolactone or they had insufficient medication data to determine a stable baseline or response window; if they did not have sufficient blood pressure measurements in the determined baseline and response periods; and finally if they did not have sufficient blood pressure measurements in both the baseline and the response periods.

4.8 Tables

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Table 12. ICD-9 and ICD-10 codes used for history of diagnosis

Diagnosis	ICD-9 codes	ICD-10 codes	Other
Chronic kidney disease stage 3	585.1	N18.1	eGFR > 30 mL/min/1.73 m^2
	585.2	N18.2	but < 60 mL/min/1.73 m^2
Ischemic heart disease	410.*	I20.*	
	411.*	I22.*	
	413.*	I24.*	
	414.*	125.*	
Type 2 diabetes mellitus	250	E11	
	250.*	E11.*	
	327.2*	G47.3*	
Smoking	305.1	F17.*	
	V15.82	Z87.891	

Abbreviations: eGFR, estimated glomerular filtration rate

Table 13. Comparison of European American patients with resistant hypertension in the SD and BioVU

Variable	SD	BioVU	p-value
Age	64.5	63.9	0.003
Female, n (%)	6615 (48.9)	3340 (49.0)	0.82
BMI	32.3	32.2	0.44
SBP	144.1	143.8	0.39
DBP	78.3	78.1	0.50
Diagnostic History			
T2DM, n (%)	2694 (19.9)	1977 (29.0)	<0.001
Sleep Apnea, n(%)	868 (6.4)	671 (9.9)	<0.001
Medication use			
Aldo antagonist, n (%)	854 (6.3)	412 (6.0)	0.48
ACE inhibitor, n (%)	6999 (51.7)	3513 (51.6)	0.87
ARB, n (%)	5178 (38.2)	2675 (39.3)	0.16
Alpha-2 agonist, n (%)	1921 (14.2)	912 (13.4)	0.12
Alpha antagonist, n (%)	626 (4.6)	283 (4.2)	0.13
BB, n (%)	8697 (64.2)	4264 (63.6)	0.02
CCB, n (%)	9272 (68.5)	4617 (68.9)	0.31
Thiazide diuretic, n(%)	8812 (65.1)	4594 (68.6)	0.001
Nonthiazide diuretic, n(%)	4271 (31.5)	2066 (30.3)	0.08
Renin inhibitor, n(%)	275 (2.0)	125 (1.8)	0.36
Vasodilator, n(%)	903 (6.7)	432 (6.3)	0.38

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BB, beta blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; SBP, systolic blood pressure; SD, synthetic derivative; T2DM, type two diabetes mellitus. Data is presented as mean for continuous variables and count (%) for categorical variables.

Table 14. Comparison of African American patients with resistant hypertension in the SD and BioVU

Variable	SD	BioVU	p-value
Age	56.3	56.7	0.27
Female, n (%)	2092 (59.1)	1020 (60.2)	0.45
BMI	34.8	34.4	0.28
SBP	149.4	149	0.60
DBP	85.6	84.7	0.06
Diagnostic History			
T2DM, n (%)	954 (26.9)	640 (37.8)	<0.001
Sleep Apnea, n(%)	252 (7.1)	159 (9.4)	0.01
Medication use			
Aldo antagonist, n (%)	240 (6.8)	101 (6.0)	0.28
ACE inhibitor, n (%)	1916 (54.1)	904 (53.3)	0.62
ARB, n (%)	1161 (32.8)	564 (33.3)	0.73
Alpha-2 agonist, n (%)	736 (20.8)	323 (19.1)	0.15
Alpha antagonist, n (%)	139 (3.9%)	59 (3.5)	0.49
BB, n (%)	1996 (56.4)	952 (56.2)	0.91
CCB, n (%)	2625 (74.1)	1234 (72.8)	0.31
Thiazide diuretic, n(%)	2319 (65.5)	1121 (66.1)	0.66
Nonthiazide diuretic, n(%)	1190 (33.6)	564 (33.3)	0.83
Renin inhibitor, n(%)	43 (1.2)	20 (1.2)	1.0
Vasodilator, n(%)	422 (11.9)	185 (10.9)	0.31

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; BB, beta blocker; BMI, body mass index; CCB, calcium channel blocker; DBP, diastolic blood pressure; SBP, systolic blood pressure; SD, synthetic derivative; T2DM, type two diabetes mellitus. Data is presented as mean for continuous variables and count (%) for categorical variables.

Table 15. Characteristics of spironolactone responders and nonresponders

Variable	Nonresponders (n=283)	Responders (n=694)	p-value
Age, years	62.84±13.39	64.85±11.93	0.04
Female, n (%)	148 (52.3%)	363 (52.3%)	1.0
Black Race, n (%)	57 (20.1%)	169 (24.4%)	0.16
BMI, kg/m^2	32.23±7.40	33.49±11.63	0.19
SBP, mmHg	133.88±17.42	148.91±19.50	<0.001
DBP, mmHg	73.09±11.81	79.76±13.36	<0.001
Diagnostic History			
CKD3, n (%)	113 (39.9%)	280 (40.3%)	0.90
IHD, n (%)	105 (37.1%)	231 (33.3%)	0.26
Smoking, n (%)	60 (21.25)	148 (21.3%)	0.97
T2DM, n (%)	152 (53.7%)	361 (52.0%)	0.63
Baseline measures			
Serum potassium, mmol/L	3.98±0.50	3.92±0.45	0.10
Serum sodium, mmol/L	138.57±3.13	139.31±3.04	<0.001
Creatinine, mg/dL	1.11±0.38	1.10-±0.41	0.43
eGFR, $mL/min/1.73m^2$	76.67±24.60	77.18±21.03	0.46
Glucose, mg/dL	124.03±60.31	124.61±47.86	0.82
HbA1c, %	7.08±2.0	8.09±14.83	0.46
HDL, mg/dL	48.62±18.60	46.94±16.26	0.49
LDL, mg/dL	98.84±40.98	99.43±36.27	0.58

Triglycerides, mg/dL	179.00±132.60	169.55±115.24	0.86
Difference from baseline following NSD			
Serum potassium, mmol/L	0.12±0.50	0.27±0.43	<0.001
Serum sodium, mmol/L	-0.42±2.53	-1.04±2.67	0.004
Creatinine, mg/dL	0.07±0.33	0.15±0.28	<0.001
eGFR, mL/min/1.73m^2	-6.63±15.26	-10.51±16.07	<0.001
Glucose, mg/dL	0.11±52.63	1.39±39.66	0.64
HbA1c, %	-0.20±1.60	0.11±1.20	0.43
HDL, mg/dL	0.60±10.86	-1.23±10.26	0.40
LDL, mg/dL	-8.98±30.72	-8.29±36.4	0.91
Triglycerides, mg/dL	-26.05±117.51	3.63±85.82	0.10

Abbreviations: BMI, body mass index; CKD 3, chronic kidney disease stage three; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; IHD, ischemic heart disease; LSL, low-density lipoprotein; SBP, systolic blood pressure; T2DM, type two diabetes mellitus.

Data is presented as mean ± standard deviation for continuous variables and count (%) for categorical variables.

Table 16. Characteristics of African American spironolactone responders and nonresponders.

Variable	Nonresponders (n=57)	Responders (n=169)	p-value
Age	56.28±11.28	60.24±12.17	0.03
Female, n(%)	43 (75.4%)	111 (65.7%)	0.19
BMI, kg/m^2	35.25±9.20	37.47±18.75	0.39
SBP, mmHg	140.08±17.91	154.65±20.76	<0.001
DBP, mmHg	79.31±11.68	86.1±13.37	0.001
Diagnostic History			
CKD3, n(%)	24 (42.1%)	70 (41.4%)	1.0
IHD, n(%)	14 (24.6%)	44 (26.0%)	0.86
Smoking, n(%)	13 (22.8%)	30 (17.8%)	0.44
T2DM, n(%)	34 (59.6%)	108 (63.9%)	0.64
Baseline measures			
Serum potassium, mmol/L	3.84±0.49	3.82±0.42	0.74
Serum sodium, mmol/L	139.37±2.52	140.09±.85	0.12
Creatinine, mg/dL	1.15±0.38	1.15±0.49	1.0
eGFR, mL/min/1.73m^2	82.73±24.65	83.04±22.90	0.93
Glucose, mg/dL	137.53±74.47	123.75±50.13	0.15
HbA1c, %	7.94±2.58	7.19±1.42	0.12
HDL, mg/dL	50.31±15.82	50.36±16.46	0.99
LDL, mg/dL	107.05±49.97	112±33.55	0.59
Triglycerides, mg/dL	159.25±73.93	134.66±79.96	0.23

Difference from baseline following NSD			
Serum potassium, mmol/L	0.18±0.50	0.24±0.36	0.34
Serum sodium, mmol/L	0.16±2.30	-0.98±0.40	0.01
Creatinine, mg/dL	0.09±0.27	0.16±0.26	0.90
eGFR, mL/min/1.73m^2	-12.03±17.25	-10.07±17.70	0.48
Glucose, mg/dL	-15.47±62.94	2.27±40.77	0.02
HbA1c, %	-0.70±2.41	0.37±1.32	0.05
HDL, mg/dL	5.11±21.74	-2.25±12.27	0.23
LDL, mg/dL	6.08±8.72	-9.64±35.99	0.30
Triglycerides, mg/dL	-18.37±59.63	1.04±55.91	0.45

Abbreviations: BMI, body mass index; CKD 3, chronic kidney disease stage three; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; SBP, systolic blood pressure; T2DM, type two diabetes mellitus.

Data is presented as mean ± standard deviation for continuous variables and count (%) for categorical variables.

Table 17. Characteristics of European American spironolactone responders and nonresponders

Variable	Nonresponders (n=226)	Responders (n=525)	p-value
Age, years	64.50±13.40	66.34±11.48	0.06
Female, n (%)	105 (46.5%)	252 (48.0%)	0.75
BMI, kg/m^2	31.47±6.69	32.21±7.70	0.21
SBP, mmHg	132.32±17.0	147.06±18.72	<0.001
DBP, mmHg	71.52±11.33	77.71±12.71	<0.001
Diagnostic History			
CKD3, n(%)	89 (39.4%)	210 (40.0%)	0.94
IHD, n(%)	91 (40.3%)	187 (35.6%)	0.25
Smoking, n(%)	47 (20.8%)	118 (22.5%)	0.63
T2DM, n(%)	118 (52.2%)	253 (48.2%)	0.34
Baseline measures			
Serum potassium, mmol/L	4.02±0.50	3.96±0.45	0.19
Serum sodium, mmol/L	138.35±3.25	139.02±3.05	0.02
Creatinine, mg/dL	1.10±0.38	1.07±0.35	0.43
eGFR, mL/min/1.73m^2	75.06±24.39	75.11±19.95	0.98
Glucose, mg/dL	120.36±55.54	124.93±47.06	0.32
HbA1c, %	6.88±1.79	8.45±17.58	0.44
HDL, mg/dL	48.20±19.27	45.72±16.06	0.27
LDL, mg/dL	96.71±38.42	94.79±36.16	0.71
Triglycerides, mg/dL	183.36±142.29	181.76±123.12	0.92

Difference from baseline following NSD			
Serum potassium, mmol/L	0.11±0.50	0.28±0.46	<0.001
Serum sodium, mmol/L	-0.58±2.57	-1.07±2.77	0.05
Creatinine, mg/dL	0.06±0.35	0.14±0.29	0.01
eGFR, mL/min/1.73m^2	-5.09±14.33	-10.67±15.45	<0.001
Glucose, mg/dL	4.51±48.66	0.65±39.26	0.33
HbA1c, %	-0.001±1.14	-0.05±1.10	0.84
HDL, mg/dL	-0.27±7.46	-0.85±9.47	0.75
LDL, mg/dL	-11.80±32.58	-7.79±36.77	0.60
Triglycerides, mg/dL	-27.20±124.36	4.59±94.81	0.12

Abbreviations: BMI, body mass index; CKD 3, chronic kidney disease stage three; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; SBP, systolic blood pressure; T2DM, type two diabetes mellitus.

Data is presented as mean ± standard deviation for continuous variables and count (%) for categorical variables.

Table 18. The p-values for significant linear correlations with change in systolic and diastolic blood pressures

	Associated Variable	PCC	Univariate	Multivariate
Total population				
Change in SBP	Change in DBP	0.67	<0.001	<0.001
	Baseline serum potassium	0.08	0.03	0.87
	Change in serum sodium	0.08	0.03	0.04
	Change in serum potassium	-0.16	<0.001	0.01
	Change in creatinine	-0.17	<0.001	<0.001
Change in DBP	Change in SBP	0.67	<0.001	<0.001
	Baseline serum potassium	0.10	0.01	0.53
	Baseline serum sodium	-0.09	0.02	0.67
	Change in serum potassium	-0.12	0.001	0.02
	Change in serum sodium	0.08	0.03	0.05
	Change in creatinine	-0.18	<0.001	<0.001
African Americans				
Change in SBP	Change in DBP	0.70	<0.001	<0.001
	Change in serum sodium	0.17	0.02	0.03
	Change in creatinine	-0.24	0.001	0.003
	Change in glucose	-0.20	0.007	0.04
Change in DBP	Change in SBP	0.70	<0.001	<0.001
	Change in serum potassium	-0.14	0.05	0.02

	Change in serum sodium	0.16	0.04	0.06
	Change in creatinine	-0.26	<0.001	<0.001
European Americans				
Change in SBP	Change in DBP	0.66	<0.001	<0.001
	Baseline serum potassium	0.12	0.004	0.73
	Baseline creatinine	0.09	0.02	0.03
	Change in serum potassium	-0.19	<0.001	0.01
	Change in creatinine	-0.15	0.001	<0.001
Change in DBP	Change in SBP	0.66	<0.001	<0.001
	Baseline serum potassium	0.09	0.03	0.68
	Baseline serum sodium	-0.10	0.02	0.60
	Baseline HDL	0.12	0.04	0.06
	Change in serum potassium	-0.12	0.01	0.12
	Change in creatinine	-0.15	0.001	<0.001

Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein; PCC, Pearson Correlation Coefficient; SBP, systolic blood pressure.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

Hypertension or high blood pressure affects approximately 45.6% of United States adults.[231] While combination therapy controls blood pressure in most patients, patients with resistant hypertension have uncontrolled blood pressure despite treatment with three or more antihypertensive medications including a thiazide diuretic. Patients with resistant hypertension have an increased risk of myocardial infarction, stroke, and renal disease.[5, 14, 15] The pathophysiology underlying resistant hypertension is inadequately understood and very few novel antihypertensive medications have been identified for the treatment of the condition. Throughout this dissertation we demonstrate that electronic medical records (EMR), such as Vanderbilt University Medical Center's (VUMC) Synthetic Derivative (SD) provide a robust research tool for the study of patients with resistant hypertension.[119]

In **Chapter 2**, we describe the development of highly accurate electronic algorithms for the identification of patients with resistant and controlled hypertension in VUMC SD. We report a prevalence of resistant hypertension of 7.3% in EA and 10.6% in AA with hypertension. We observe that the characteristics of patients with resistant hypertension identified by the electronic algorithms are similar to those previously reported in epidemiologic studies or clinical trials of resistant hypertension. Patients with resistant hypertension are older, heavier, more likely to have chronic kidney disease stage 3, and more likely to have type 2 diabetes mellitus and sleep apnea than controlled hypertensives.[5, 135-138] While the identified population was similar to populations previously reported, the lower prevalence of sleep apnea, diagnosed by International Classification of Disease (ICD-9 and -10) codes, compared to those estimated in studies where

sleep apnea is prospectively defined suggests the need to improve the clinical diagnosis of sleep apnea.[139-141]

In **Chapter 2** the availability of a large number of patients with resistant hypertension in the EMR allowed us to identify trends in antihypertensive prescribing based on race. The use of aldosterone antagonists such as spironolactone increased from approximately 13% to 40% with time in both racial groups. These rates may be relatively low for RH patients in light of evidence for the efficacy of aldosterone receptor antagonists.[48, 51, 124, 159-162] Ultimately, these findings have the potential to inform clinical prescribing practices.

In addition to identifying patient characteristics associated with resistant hypertension, studies in EMRs linked to genetic material, including VUMC's BioVU and the Department of Veterans Affairs (VA) Million Veterans Program (MVP), have the potential to explore the genetic underpinnings of resistant hypertension.[117, 119] In **Chapter 3** we demonstrate the use of the MVP to perform a genetic association study to evaluate a potential novel mechanism underlying resistant hypertension.

Because salt-sensitivity is frequent in patients with resistant hypertension and studies in rats suggest that genetically decreased 20-hydroxeicosatetraenoic acid (20-HETE) promotes salt-sensitive hypertension we tested the hypothesis that variants in *CYP4A11*, the gene encoding the enzyme responsible for the metabolism of arachidonic acid to 20-HETE in humans, are associated with resistant hypertension.[169, 173, 223, 232] In **Chapter 3** we identified a statistically but not clinically significant association between *CYP4A11* loss-of-function variants, rs1126742 and rs3890011, and resistant hypertension in the total population. It is possibly, however, that these variants may play a larger role in a subset of the population based on clinical or other genetic factors. Future work to replicate these findings in a second biorepository is necessary to confirm

the association. In addition there is a need to identify other novel pathways using an agnostic approach.

Finally in **Chapter 4** we demonstrate the development of a highly accurate algorithm for the identification of blood pressure response to spironolactone. The mean decreases in systolic blood pressure (8.5±18.1 mmHg) and in diastolic blood pressure (4.0±9.9 mmHg) were similar to those published previously.[51, 198, 201] We estimated that spironolactone lowered blood pressure within six month in 71% of resistant hypertensive patients treated with spironolactone. The decrease in blood pressure after the start of spironolactone correlated with decreases in serum sodium and increases in serum potassium consistent with decreased epithelial sodium channel (ENaC) activity. This may suggest that nonresponders to spironolactone may have increased ENaC activity, a hypothesis that needs to be tested. In European Americans and the total population, responders had larger increases in serum creatinine and larger decreases in estimated glomerular filtration rate (eGFR) after spironolactone start than nonresponders. In African Americans, responders had a significant increase in glucose and a decrease in systolic blood pressure correlated with an increase in glucose.

The latter finding is particularly interesting in light of rodent data suggesting that aldosterone decreases insulin secretion through a mineralocorticoid-receptor (MR) independent mechanism.[215] During MR antagonism aldosterone concentrations increase due to loss of feedback inhibition and aldosterone concentrations may be higher in blacks compared to withes. Some clinical trials have reported an increase in hemoglobin A1c in diabetic patients and patients with heart failure treated with spironolactone.[212, 233, 234]

Future work will include the incorporation of the *CYP4A11* genetic variants, rs1126742 and rs3890011, for the resistant and controlled hypertension populations in BioVU. When

genetic data is available we will be able to test the association between these variants and resistant hypertension in the BioVU population, thus serving as a replication for the findings in **Chapter 3**. We will also be able to test for an association between these variants and spironolactone response using the population identified in **Chapter 4**.

While our work demonstrates how EMRs may be used to study resistant hypertension the work is not without limitations. The algorithms for identification of resistant hypertension in both the SD and MVP use blood pressures collected in an outpatient clinic, not from an ambulatory blood pressure monitoring device, so it is impossible to exclude individuals with white-coat hypertension. Further, while these datasets incorporate refill data in to their medication records it is impossible to confirm medication adherence without testing a patient's plasma for the prescribed medications or urine for metabolites. Future work to incorporate ambulatory blood pressure measurements and direct measures of medication levels in to patient records will be important to identify the patients in the population with true resistant hypertension.

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