Pharmacogenomics in hypertension: Where we stand today

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Abstract: Hypertension is a common and growing medical problem that leads to enormous cardiovascular and kidney disease worldwide. While many drugs exist to treat hypertension, there is large individual variation in how a given individual may respond to different agents, which contributes to dismal rates of hypertension control. While demographic factors predict which drugs may work better in certain individuals, a great degree of this variation has a genetic basis. In recent years, genome wide association studies have begun to identify specific gene variants that predict drug response to particular agents. This review identifies the major genetic variants influencing antihypertensive response that have emerged from this growing body of work. For novel genetic variants without a previously known biologic basis in blood pressure, it is crucial to validate initial findings in subsequent studies. This information may eventually lead to a more personalized approach to hypertension management that will improve blood pressure control and patient outcomes. The integration of this large amount of data and its real world application will be highly challenging, but strategies to accomplish this are discussed.

Keywords: blood pressure, diuretics, thiazide, beta blocker, calcium channel blocker, renin angiotensin system
Introduction

Hypertension (HTN) is responsible for significant morbidity and mortality across the United States, and indeed, worldwide. As the prevalence of obesity increases, the disease burden of hypertension will only increase [1]. The underlying pathophysiology of hypertension is complex and varies significantly between patients, which makes HTN challenging to treat successfully. There are multiple choices of drugs and drug classes to treat hypertension, and yet the rate of successful blood pressure control is disappointing, typically less than 50%, due in part to great variability between patients as to which drugs are effective. Choices of what antihypertensive class to choose can be guided partially by demographic factors, with diuretics and calcium channel blockers generally more effective in African Americans and the elderly, and beta blockers and drugs targeting the renin-angiotensin system (RAS) more effective in Caucasian and younger patients [2]. Nevertheless agents are often selected in a trial and error fashion. The hit-or-miss nature of this approach is inherently inefficient and contributes to greater medical costs, delayed BP goal attainment, poorer BP management success rates, and continued high cardiovascular mortality. Predictive models or genetic information which could help identify those agents that would be most effective in a given patient would be of immense clinical value, improve BP control, and would undoubtedly reduce the consequences of hypertension and save medical costs.

Increasingly the field of pharmacogenomics has been used in areas such as cardiology and oncology to inform clinicians which drugs may have greater or lesser effectiveness as well as potentially greater risk profiles of adverse effects. The pathogenesis of elevated BP and HTN is a complex interplay of environmental and multiple genetic factors which may differ greatly between
individuals. The genetic basis of HTN is based on both rare Mendelian regulation of BP as well as heritability estimates of BP in mono and dizygotic twins in shared environments demonstrating heritability of up to 50% [3]. Several autosomal recessive monogenic causes of HTN that have been identified, such as Gordon’s syndrome, which results from mutations in the WNK kinase genes, leading to increased renal sodium reabsorption, and Liddle’s syndrome, which is caused by mutations in genes SCNN1B or SCNN1G which lead to increased activity of the distal ENaC sodium channel, also leading to increased sodium retention by the kidney [4,5]. Additionally, a number of genetic causes of aldosterone excess or hypersensitivity to corticosteroids have been identified, such as mutations in CYP11B2, CYP11B, HSD11B2, and KCNJ5 [6-9]. Although the individuals affected by these syndromes are often severely hypertensive, these single mutations with a Mendelian effect are extremely rare. The vast majority of patients with essential HTN have genetic contributions to their BP, but are more representative of a complex medical disorder where multiple gene variants which each have a small contribution to the BP phenotype [10], in the setting of interaction with environmental factors. There are likely hundreds of genetic variants which play a role, interacting with other variants that may be protective against hypertension.

One approach to identifying genetic variants associated with differential response to particular BP agents is to focus on the effect of candidate genes previously identified as having a mechanistic role in hypertension. This approach relies on prior knowledge of blood pressure regulatory systems and their genetic influences. Given that our knowledge of these systems is not complete and that there are other as of yet unknown regulators of each BP regulating system, significant bias is present in these types of studies, and both negative and positive findings can only be interpreted in a limited
context. In contrast, a genome wide association study (GWAS) pharmacogenomic approach will identify completely novel SNPs which correlate with a greater or lesser response to specific BP agents. After the necessary statistical correction for the very large number of multiple comparisons of hundreds of thousands of SNPs tested in each patient using current gene arrays, no SNPs have emerged with an incontestable association with BP response to any antihypertensive agent ($p < 10^{-8}$). This is not surprising given the modest number of patients that can be studied in randomized trials, in contrast to the thousands of subjects analyzed in GWAS for incident HTN or other common diseases. However, multiple suggestive SNPs with $p$ values in the $1 \times 10^{-5}$ to $1 \times 10^{-6}$ range have been identified in various studies. Regardless of the strength of the genetic associations, validation populations are needed to confirm candidate SNPs that have been identified. Such an unbiased approach using a GWAS of BP response to specific antihypertensive agents has successfully identified and validated clinically relevant variants that could not have been predicted based on our current understanding of the physiology of BP regulation [11-13]. In addition to cross-validation studies, as the cost of DNA sequencing technology has fallen significantly, multiple single studies have been performed describing associations of various SNPs, but either remain unvalidated, or subsequent validation attempts have failed to confirm an effect. This review will focus on gene variants in which the evidence for a significant effect on the response to antihypertensive agents is the strongest, which emerge from high quality, well powered studies with subsequent validation in separate cohorts. A summary of important hypertension cohorts used in these pharmacogenomics analyses is given in Table 1.
### Table 1. Major hypertension pharmacogenomics studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects (n)</th>
<th>Population</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORDIL</td>
<td>3863</td>
<td>Norway, Sweden</td>
<td>HCTZ / atenolol / diltiazem</td>
</tr>
<tr>
<td>INVEST</td>
<td>5598</td>
<td>American - multiethnic</td>
<td>Atenolol / verapamil</td>
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<tr>
<td>PEAR1</td>
<td>768</td>
<td>European Americans, African Americans</td>
<td>HCTZ / atenolol</td>
</tr>
<tr>
<td>PEAR2</td>
<td>457</td>
<td>European Americans, African Americans</td>
<td>Chlorthalidone / metoprolol</td>
</tr>
<tr>
<td>GERA1</td>
<td>505</td>
<td>European Americans, African Americans</td>
<td>HCTZ</td>
</tr>
<tr>
<td>GENRES</td>
<td>228</td>
<td>Finland</td>
<td>HCTZ / bisoprolol / amlodipine / losartan</td>
</tr>
<tr>
<td>HCTZ Milan</td>
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<td>Italy</td>
<td>HCTZ</td>
</tr>
<tr>
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<td>465</td>
<td>Italy</td>
<td>HCTZ</td>
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<tr>
<td>LIFE</td>
<td>3503</td>
<td>Norway, Denmark, Finland</td>
<td>Atenolol / losartan</td>
</tr>
<tr>
<td>AASK</td>
<td>328</td>
<td>African Americans with chronic kidney disease</td>
<td>Metoprolol</td>
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Abbreviations: NORdic DILTiazem Study; INternational VErapamil Trandolapril Study; INternational VErapamil Trandolapril Study; Pharmacogenomic Evaluation of Antihypertensive Responses 1; Pharmacogenomic Evaluation of Antihypertensive Responses 2; Genetic Epidemiology of Responses to Antihypertensives 1; GENes and Antihypertensive Drug RESponse; Milan Hypertension Pharmacogenomics of HCTZ; Pharmacogenomics of Hydrochlorothiazide Sardinian Study; Losartan Intervention For Endpoint reduction in Hypertension; African American Study of Kidney Disease and Hypertension
Diuretics

Diuretics, usually of the thiazide class which targets the distal convoluted tubule, are one of the most common initial agents in treatment of HTN, and currently recommended as first line therapy by recent JNC 8 and ACC/AHH guidelines. Importantly, the BP response to this class of antihypertensive agents is quite uneven \[2,14,15\]. Much has recently been learned about the some key genetic influences on thiazide diuretic BP response, from both candidate driven and GWAS approaches. Variants in \textit{NEDD4L} (neural precursor cell-expressed developmentally downregulated 4-like), were previously found to have a role in incident hypertension \[16-18\], and so emerged as an attractive candidate for pharmacogenomic study. \textit{NEDD4L} controls the protein expression of the renal distal tubular sodium channel ENaC, as well as other sodium channels, through ubiquitination. Variation at the intronic SNP rs4149601 produces a cryptic slice site and leads to greater expression of ENaC and a hypertensive, low renin phenotype, which has been linked to increased cardiovascular mortality \[19\]. The NORDIL study (NORdic DILtiazem Study), whose participants were overwhelmingly Caucasian, found the carriers of the G allele to be associated with a greater decrease in SBP and DBP when treated with thiazide diuretics, but not CCBs, as would be expected \[20\]. This result was replicated by the PEAR1 study in a Caucasian population \[21\]. Additionally, carriers of the G allele were found to have better cardiovascular outcomes in the NORDIL population (OR 0.52, 95% CI 0.36-0.74) when treated with thiazides \[20\], and the INVEST cohort (INInternational VErapamil Trandolapril Study) similarly found higher mortality in G allele carriers who were not treated with thiazides. The latter study also found that addition of a second SNP in the 5′ untranslated region of \textit{NEDD4L}, rs292449, improved the prediction model for SBP and DBP when analyzed as part of a haplotype with rs4149601. In summary, the clear biologic role of \textit{NEDD4L}, the consistent findings
of these several studies, and the association with longer term outcomes in addition to short term BP response makes the pharmacogenomics implications of this gene very robust. It is worth noting however that these findings were from Caucasian populations and did not replicate in African Americans within the PEAR1 study (Pharmacogenomic Evaluation of Antihypertensive Responses 1), which is important given that hypertensive African Americans are more volume expanded and salt sensitive, with lower circulating plasma renin activity, and generally show more BP response to thiazide diuretics in comparison to Caucasians.

An example of pharmacogenomics markers of BP response in hypertensive African Americans was identified by an unbiased approach as opposed to a candidate gene approach shown by the association of three SNPS on the chromosome 12q5 region, rs317689, rs315135, and rs7297610, in the GERA1 study (Genetic Epidemiology of Responses to Antihypertensives 1). These SNPs are outside protein coding regions but in the vicinity of the genes for LYZ (lysosome), FRS2, and YEATS4. The strongest association was seen with rs7297610 near YEATS4, where the C allele correlated with greater BP response to HCTZ [22]. This finding was validated in hypertensive African American participants in a separate cohort from the PEAR1 study (SBP and DBP response -13.0 and -8.0 in the CC genotype, versus -9.6 and -5.2 mm Hg in the TT genotype). YEATS4 had no previously known role in BP regulation, but it codes for the protein GAS41, a transcription factor which modifies gene expression through acetylation of histones H4 and H2A, and may participate in inflammatory signaling [23]. Support for a role in HTN and HCTZ response was strengthened by the subsequent finding that leukocytes in the CC variant participants had statistically greater YEATS4 expression at baseline (p= 0.009) and a statistically greater decrease in expression (p=0.02) after HCTZ treatment,
which was not observed in carriers of the T allele [11]. While the pathophysiology behind this mechanism has not been uncovered yet, the independent validation and changes in YEATS4 expression level strengthen these findings; thus unexpected GWAS findings may eventually lead to a greater understanding in pathophysiology.

A separate unbiased analysis of a combined Caucasian cohort from the GERA1 and PEAR1 studies identified suggestive (P < 1 x 10^{-5}) associations between BP response to thiazides in several SNPs, and then tested these candidates for replication in a larger cohort from the GENRES (GENes and Antihypertensive Drug REsponse), NORDIL, and the HCTZ-Milan (Milan Hypertension Pharmacogenomics of HCTZ) studies. Validation was found for two of these initially suggestive SNPs [13]. rs16960228 is located in an intron within PKCA (protein kinase Cα). Meta-analysis of these cohorts found a p value of 3.3 x 10^{-8}, which meets the commonly accepted threshold for genome wide significance testing of 10^{-8}. This variant had an opposite direction of effect for atenolol within the PEAR1 cohort, consistent with the partially opposing mechanisms of action of these two classes of drugs. Animal studies in PKCA knockout mice have recently shown BP differences attributable to altered vascular contractility [24]. Additionally, other SNPs in this gene were also found to correlate with DBP sensitivity to thiazides in an African American cohort of PEAR1. This same analysis also found that variation in rs2273359, located between genes GNAS (stimulatory G-protein α subunit) and EDN3 (endothelin 3), correlated with SBP response to thiazides [13], with a p value of 5.5 x 10^{-8}. The region near GNAS is highly complex, involving several alternative promotors and variant transcripts, with animal work also suggesting a role in BP regulation and the adrenergic nervous system [25,26].
A number of other reports have found gene variants associated with differential responses to thiazide diuretics, implicating variants in TET2, CSMD1 [27], GNB3 [28], and VASP (vasodilator associated phosphoprotein) [29]. While these variants have not been as consistently validated [30] and did not obtain $p$ values $< 10^{-8}$, they do have biologic rationale as an aldosterone sensitive regulator of ENaC (TET2), a prior association with vascular disease and HTN (CSMD1), an association with HTN and renin levels (GNB3), or a prominent role in vascular biology (VASP). Studies showing variation at rs10995, downstream of VASP, were also interesting in that they not only predicted better blood pressure response in two independent cohorts, but also associated with the baseline level of VASP mRNA [29]. Thus the number of genes that may influence diuretic response is continuing to grow. As each genetic variant may have a relatively small influence, the challenge is to accurately integrate this information to get a more global idea of how an individual patient may respond to a certain antihypertensive drug class. An illustration of this is the combination of multiple data sets in a GWAS of HCTZ response in a combined cohort of over 1700 patients from the GENRES, GERA1, HCTZ-Milan, PHSS (Pharmacogenomics of Hydrochlorothiazide Sardinian Study), NORDIL, and PEAR1 studies. This meta-analysis did not find any Bonferroni-corrected variants of significance, but did find two suggestive ($P < 10^{-5}$) SNPs which correlated with response to HCTZ, and which were validated the African American group from the same studies [31]. rs177848 and rs177852 (in Caucasians and African Americans respectively) are located near FOXA1, which encodes a transcription factor, Forkhead Box A1, expressed in the collecting duct of the kidney, where it could play a role in water and solute handling [32]. Also identified in this analysis were rs11750990 and rs1049911 near GJA1, which encodes
connexin 43, which functions in electrochemical coupling in myocardium and vascular smooth muscle. Lastly, a parallel survey of HTN candidate genes from this meta-analysis also implicated a series of SNPs near \textit{HSD3B1}, whose gene product has a role in aldosterone synthesis [31].

**Inhibitors of the renin-angiotensin system**

The renin angiotensin system (RAS) plays a key role in BP regulation and the pathophysiology of HTN, and understandably this well-defined pathway has attracted early and ongoing attention in candidate gene studies as to whether genetic variation predicts response to a variety of antihypertensive classes, especially angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin receptor blockers (ARBs). Initial interest was focused on a polymorphism of the angiotensin converting enzyme (ACE) gene, in which the insertion (I) variant in intron 16 (mean allele frequency (MAF) approximately 45\%) has been associated with a significantly lower serum ACE level than the corresponding deletion (D) polymorphism [33]. Although initially several small and older studies suggested that those with the II genotype, identified by SNP rs1799752, had a stronger ACEI and ARB BP response [34-37], multiple larger and more recent studies have suggested there is actually no relationship [38-43]. Likewise, the literature has also failed to consistently show a role for variants of angiotensinogen (\textit{AGT}) or variants of angiotensin II receptors \textit{AGTR1} or \textit{AGTR2} [44]. However, there have been two separate studies in Asian populations showing that variants in the renin (\textit{REN}) gene affect BP response to valsartan. Here the CC genotype of the C-5312-T polymorphism was associated with greater DBP response, as well as a smaller compensatory increase in serum renin levels after treatment, which lends some biologic plausibility to these results [40,45,46].
More recently, an intriguing finding from the GENRES study of in Finnish men randomized to several different antihypertensive drugs found in an unbiased genome analysis that carriers of a C allele at rs3814995 had greater BP response to losartan [47]. This class effect was replicated in American and European cohorts, and also in a separate analysis of Finnish patients in the LIFE (Losartan Intervention For Endpoint reduction in Hypertension) trial [48]. rs3814995 is a SNP within NPHS1, where the A allele codes for a missense mutation from glutamine to lysine at amino acid 117 in nephrin, a crucial protein in the glomerular slit diaphragm. This variant is considerably more common in Caucasian versus African American populations (MAF 0.30 versus 0.09) and mutations in this gene are responsible for one type of congenital nephrotic syndrome of the Finnish type [49]. The biological effect of this mutation on BP regulation has not been explored, but is interesting given the fact that ARBs have the clear effects on glomerular hemodynamics. Overall however, given the considerable knowledge about the physiology of the RAS, it is surprising that so few genes have been found to be relevant for predicting BP response to ACEIs and ARBs.

**Beta blockers**

Data supporting the influence of several genetic variants on the response to beta blockers is intriguing. The gene ADRB1 codes for the β1 receptor, and two SNPs within this gene were originally identified in population studies as a risk factor for HTN [50]. The A allele at rs1801252 leads to a Ser49 variant, which leads to increased receptor internalization, while the C allele at rs18011253 results in the Arg389Gly variant, which causes less downstream signaling to adenylyl cyclase. Multiple studies have shown that the Arg389 and/or the Ser49Arg389 haplotype are
associated with significantly better blood pressure response to beta blockers [51-54]. Importantly, analysis of the INVEST trial found that participants with the Ser49/Arg389 haplotype was associated with higher mortality when treated with a verapamil based strategy, as opposed to a beta blockers [55]. This validation in clinical outcomes, in concordance with a greater BP effect and firm biologic role, makes the case for this genetic variant highly persuasive. Nevertheless, some smaller studies, as well as analysis of the above SNPs in a 1729 patient cohort in the LIFE trial, have not supported a role for ADRB1 in BP control [41,56,57]. In contrast, studies of polymorphisms in or near the β2 receptor ADRB2, which has less expression in the heart, but more widespread distribution in the vasculature, have shown a less convincing and weaker relationship with response to beta blockers [41,58-60].

Another gene important in β1 receptor function is G protein-coupled receptor kinase 4 (GRK4), a kinase which restores β1 receptor activity after dephosphorylation by regulatory G kinases. Polymorphisms in GRK4 (rs2960306 and rs1024323) were initially found in an analysis of the AASK study (African American Study of Kidney Disease and Hypertension) to influence the time to BP control with metoprolol, although this was seen only in men [61]. The AASK trial enrolled African Americans with hypertensive nephrosclerosis and thus by design focused on individuals with poor kidney function. This finding was clarified in a mixed race populations in the PEAR1 study, which had excluded individuals with abnormal kidney function [62]. Here the number of copies of the 65Leu142Val haplotype of rs2960306/rs1024323 correlated with decreased DBP responsiveness to atenolol (-9.1, -6.8, and -5.3 mm Hg in those with 0, 1, and 2 copies), an effect present in either gender. Furthermore the 486Val variant (rs1801058) was found in the INVEST trial to correlate with worse
cardiovascular outcomes (OR 2.29, 95% CI 1.48 – 3.55), although this was seen in those getting either atenolol or verapamil.

Beyond the above genes which have a known biologic mechanism in adrenergic signaling, several other genes have emerged from unbiased analyses. One key study identified two SNPs which influenced response to beta blocker monotherapy in a combined African American cohort from the PEAR1 and PEAR2 studies. These SNPs were successfully validated in a second group of patients from PEAR1 in whom atenolol was added after HCTZ. rs201279313, a deletion of TTA in an intron of SLC25A31, was associated with a greater DBP response (p=2.5 x 10^{-8} in combined meta-analysis) [12]. This gene codes for a mitochondrial membrane ATP/ADP transporter. This study also found that the deletion variant of rs11313667 correlated with better SBP response to beta blockers (p=7.2 x 10^{-8}). rs11313667 is intronic to the gene LRRC15, which encodes leucine rich repeat containing 15, a glycoprotein normally associated with placenta and cancers. Although the biologic basis of this effect is unknown, it could also be mediated via nearby genes, such as GP5 (platelet glycoprotein V), which mediates the adhesion platelets to the endothelium and has been reported to be elevated in HTN [63].

Another example of the use of an unbiased GWAS to discover a gene influencing BP response is the gene PTPRD. Analysis of the Caucasian cohort of patients from the PEAR1 study found that SNP rs12346562, located upstream of PTPRD, correlates with improved BP response to beta blocker (p=3.2 x 10^{-6}), with an opposite direction of effect seen in HCTZ [64]. This finding was confirmed in the Finnish population of the GENRES study; rs12346562 was also found to be linked with resistant
HTN in the INVEST participants. *PTPRD* codes for receptor-type protein tyrosine phosphatase receptor delta, which has been found to have roles in neuron growth and guidance as well as malignant glioma, although through its involvement in the STAT3 pathway could possibly play a role in intrarenal RAS signaling [65]. Further support for this is given by the identification of a SNP rs10739150 downstream of *PTPRD* that correlates with BP responsiveness in the African American group from this study (p=8.3 x 10^-6).

The Caucasian cohort from the PEAR1 study was analyzed to determine if a selected set of 37 SNPs previously associated with baseline HTN predicted BP response to atenolol or HCTZ. While none of these SNPs achieved genome wide significance, six did reach nominal significance. Of these, rs1458038, near the *FGF5* gene (Fibroblast Growth Factor 5), had the most powerful effect. The T allele of rs1458038 was significantly associated with better BP response to atenolol, while being associated with decreased response to HCTZ [66]. The relationship of this SNP to HTN at baseline in prior studies, the opposite directions of effect in different drug classes, and the strong linear relationship between the number of T alleles and both SBP and DBP response all support a likely association. This analysis additionally combined several others of these significant SNPs to calculate genetic scores accounting for 8.5% and 4.3% of the variation in SBP response to atenolol and HCTZ respectively. Of note, none of these variants had significant associations in the African American population within PEAR1. This level of prediction for BP response is considerably stronger than what has been observed for a risk score explaining the degree of untreated BP at baseline, which was estimated to be approximately 2.2% using the combined effect of 116 independent loci [67].
Calcium channel blockers (CCBs)

Less work has directly examined the genetic predictors of CCB antihypertensive effect. However, longer term studies of cardiovascular outcomes such as the INVEST study has given us valuable information about the genetic influence on the ability of CCBs to reduce mortality. This study was an international multiracial comparison of verapamil versus atenolol in patients with HTN and CAD. The gene KCNMB1 encodes the beta 1 subunit of the BK channel, and mutations within this gene were previously known to lead to HTN and left ventricular hypertrophy [68-70]. Thus variants of this gene were examined as to the effect on cardiovascular outcomes [71]. Carriers of rs11739136, which causes a glu65lys translocation in KCNMB1, achieved target BP control faster when treated with verapamil, but not atenolol. Carriers of rs2301149, which have a val110leu translocation in KCNMB1, had significantly lower rates of nonfatal MI when treated with verapamil, but not atenolol. Thus two nonsynonymous SNPs from this gene are associated with altered responsiveness to verapamil.

The gene CACNA1C encodes the alpha1C subunit of the L-type calcium channel, which is strongly expressed in heart and vascular smooth muscle and is the main target of calcium channel blockers. Eight variants of this gene were examined in the INVEST cohort. Of these, variation at rs1051375 correlated with outcomes, specifically a composite of death, nonfatal myocardial infarction, and nonfatal stroke [72]. The AA haplotype of this SNP had a reduction in composite primary outcomes to a hazard ratio of 0.54 (95% CI 0.32-0.92), while the GG haplotype had a hazard ratio of 4.59 (95% CI 1.67-12.67). The combination of previously known biologic relevance and the demonstration that these variants have relatively large effects on long term patient outcomes makes
these findings highly relevant. The functionally related gene CACNB2, which encodes the regulatory beta-2 subunit of the voltage gated calcium channel, was also suggested to have a role in an analysis from INVEST. The GG haplotype of rs3129507, located within the promoter region of CACNB2, was associated with an increase in adverse cardiovascular outcomes in the verapamil group (HR 2.35, 95% CI 1.19-4.66, p = 0.014) [73]. A second SNP in this gene, rs11014166, was associated with outcome differences within the Hispanic subpopulation of this study. While BP response was not directly tracked as an outcome measure in these studies, the differences in outcome seen only in the verapamil treated group suggest a genetic basis for differential sensitivity to calcium channel blockers with these variants.

While the above findings were all directed at biologically based candidate genes, one study of a Caucasian cohort from the INVEST study used a GWAS of nonsynonymous SNPs to develop a risk score to identify differential mortality benefits with a CCB versus beta blocker treatment [74]. The derived risk score was based on the additive effect of three novel variants: rs16982743, rs893184, and rs4525, respectively located within SIGLEC12, A1BG, and F5. This score ranged from 0-3; a score of 0-1 was associated with improved outcomes in those treated with a CCB (OR 0.60, 95% CI 0.42-0.86), while a score of 2-3 was significantly associated with worse cardiovascular outcomes with a CCB versus a beta blocker (OR 1.31, 95% CI 1.08-1.59). This initial identification of these three SNPs was validated in an analysis of the NORDIL cohort. While these genes do not yet have any known biologic role in BP regulation, other SNPs in F5 have been associated with baseline HTN [75]. This approach of combining the effect of multiple SNPs in a cumulative risk score may be an attractive approach in
the future to personalize treatment recommendations for individual patients despite the modest affect size of individual genes.

**Pharmacokinetic influences**

In addition to candidates based on mechanism of action, the response to several BP drugs is known to be influenced by genes influencing their metabolism. Losartan is a prodrug metabolized by cytochrome P450 enzymes CYP2C9, and to a lesser degree CYP3A4, to its active form [76]. Variants in CYP2C9 have been found which are associated with markedly slow conversion of losartan to its active metabolite, with significantly reduced effects on blood pressure and proteinuria [41,45,77]. Metoprolol is primarily metabolized by CYP2D6, which has multiple variant alleles, some of which are nonfunctional or hypofunctional [78,79]. Individuals carrying hypofunctional alleles have been found to have higher metoprolol blood levels, as well as differences in diastolic blood pressure (DBP), heart rate, and QT interval [80,81]. Additionally increased copy number of CYP2D6 can cause some individuals to be rapid metabolizers of metoprolol [82]. Based on this, the Dutch pharmacogenomics working Group (DPWG) has formally recommended CYP2D6 testing with metoprolol [83]. We have an incomplete knowledge of genetic influences on the pharmacokinetics of most antihypertensive drugs, but variations in drug levels may not always translate into meaningful differences in drug effect. An example is carvedilol, which is also metabolized by CYP2D6, with resultant differences in carvedilol drug levels, although differences in BP or other clinical effects have not been consistently observed [60,84]. Amlodipine and verapamil are known to be metabolized by CYP3A4 and CYP3A5 [85,86]. Several gene variants in the former were associated with better BP control with amlodipine in women in the AASK trial [85]. Lastly,
Hydralazine metabolism is affected by variants in its phase II metabolizing enzyme N-acetyltransferase-2. Individuals carrying NAT2*5, *6, and *7 alleles, present in up to 90% of North Africans and 50% of Caucasians, are more likely to have high drug levels and greater blood pressure response [87,88].

**Adverse effects**

In addition to immediate and longer term effectiveness of antihypertensives, some work has been directed at uncovering genetic predictors of known adverse effects from these drugs. Single studies have implicated potential variants that predict HCTZ-induced hypokalemia, hyperuricemia, or dyslipidemia with HCTZ or atenolol [89-91]. These studies have not yet been validated, but one study examining a panel of 33 candidate genetic variants previously associated with fasting glucose in the general population found that rs340874, located upstream of PROX1, was associated with atenolol induced hyperglycemia with a Caucasian cohort of the PEAR1 study [92]. PROX1 encodes prospero homeobox 1, which has a role in embryologic development of liver and pancreas, and may be involved in liver gluconeogenesis [93]. Additionally, an association between thiazide induced hyperglycemia and variants in renal potassium channel KCNJ1 was also suggested in an analysis of several candidate genes [94]. Subsequently, an analysis the African American cohort of the PEAR1 study found that variation at rs17137967, located within an intron of KCNJ1, was associated with thiazide induced new onset diabetes, subsequently confirmed in a multiracial group from the INVEST study [95]. Other work has explored predictors of the common problem of ACE-I induced cough, based not on a GWAS approach but by focus on genes with possible biologic rationale. An earlier finding that differences in the promotor region of bradykinin B2 receptor (BDKRB2) has not
been repeated by other groups [96-98]. More recently however, two SNPs within introns of \textit{BDKRB2} have been implicated in ACE-I induced cough, as well as findings that variance at rs495828 upstream of the \textit{ABO} gene can predict this adverse event [99-101]. Identification of patients at risk of adverse effects from antihypertensive drugs has not been nearly as well studied as genetic influences on drug efficacy, but could have a major impact on patient care and health care expenses.

\textbf{Where do we go from here?}

The above studies show the large and growing amount of knowledge we have gained about how genetic variation affects individual response to individual antihypertensive drugs. Several limitations in the growing literature on HTN pharmacogenomics remain however. The nature of BP itself makes it a challenging outcome measure to study, since it is constantly changing, and affected by diet, activity, posture, psychological stress, and a variety of other factors. This has been most notably identified in large hypertension registries from Spain and other European countries where home, office, and 24 hour ambulatory BP measures configure different BP profiles [102,103]. Some studies, such as the PEAR trials, have make a thorough effort to address this by tracking BP taken by patients manually or by automated cuff at home. Of course, short term BP measurement is a surrogate endpoint, while the morbidity and mortality of patients is our ultimate concern. Trials such as INVEST and NORDIL have addressed this and given us key data on the influence of genetic variation on long term outcomes.

The generalizability of these findings is limited by the exclusion of sicker patients with more complex comorbidities in these trials. For instance, with the exception of the AASK trial, little
pharmacogenomic data exist in the significant proportion of hypertensive patients with chronic kidney disease. As well, patients with resistant HTN (requiring more than three drugs for control) is another important group that may behave differently that what the above trials suggest, although some recent work is beginning to address this [104-106]. The identification of some novel genes also advances our understanding of human biology. Furthermore, another limitation to the GWAS approach is that using genetic arrays of known SNPs by definition excludes rare variants with allele frequencies < 0.05, thus inevitably the influence of rare but potentially powerful variants will not be detected. This may change as the cost of whole exon or whole genome sequencing becomes more feasible in the near future. Perhaps the greatest challenge is simply bringing together the vast amount of genetic information these many studies have now generated. Increasingly, work has combined cohorts from multiple studies to increase overall statistical power. While inevitably there are concerns about heterogeneity between different studies, this is clearly a step forward. The ICAPS (International Consortium for Antihypertensives Pharmacogenomics Studies) consortium, founded in 2012, has been a key force in advancing this work. ICAPS now has 29 clinical trials with 345,000 participants’ data available for analysis. These better powered analyses allows calculation of risk scores using weighted combinations of multiple allelic variants which can better predict the response to different blood pressure agents [74,107]. Inclusion of other demographic or clinical data in such a score could further enhance accuracy of these models.

Despite the valuable information obtained through the work in this field, translating this into clinical practice to improve treatment of HTN remains a challenge. Reaching expert consensus in clinical guidelines requires weighing clinical benefits, potential adverse effects, and cost. While
pharmacogenetics testing has obvious appeal in diseases like cancer, this is a situation where drug
efficacy may have immediate impact on short term survival and drug toxicity is potentially quite high. CPIC (Clinical Pharmacogenetics Implementation Consortium) is an organization which
reviews pharmacogenetic literature and makes recommendations regarding the use of this data in
medical practice. As of now CPIC does not recommend any pharmacogenomic testing in the
treatment of HTN. While the individual impact of genetic information to guide hypertensive
treatment may not always make a vast difference to individual patients, the total impact is amplified
by the vast number of patients with HTN.

One clear obstacle is the cost of genetic testing. While advancing technology has made genetic
testing vastly less expensive than even five years ago, obtaining pharmacogenetics information on
individual patients will still ultimately require insurance coverage for the vast majority of cases.
Another impediment is that when genetic information becomes routinely available, individual
physicians may vary widely in their comfort level interpreting and actually using pharmacogenomic
data to make treatment decisions for their patients. Many current physicians had their medical
training in an era before the sequencing of the human genome was achievable, and medical curricula
did not emphasize medical genetics outside the handful of monogenic diseases. Explaining to
patients the rationale and use of genetic testing presents unique challenges. Without an
understanding, familiarity, and trust of new types of testing, providers may continue their current
practice of prescribing antihypertensives based on trial and error. Thus it may be more realistic for
clinical HTN specialists at academic medical centers to be the first group of physicians to implement
pharmacogenomic information. Patients with difficult to control HTN are a subset in whom this approach may be the most natural, as well as being a group who may have the most to gain [104].

Despite these difficulties, a streamlined pharmacogenomic approach to get hypertensive patients on the most effective, efficient, and well tolerated drug regimen would have great benefits. This would result in fewer patient visits to readjust BP medications, fewer drugs per patient, and likely better compliance with their medical regimen. Better BP control would doubtless lead to fewer cardiovascular and renal complications and thus great improvement in quality of life and longevity [108-110]. Given the vast numbers of patients with HTN, this would as well result in enormous cost savings, with one recent economic analysis estimating national cost savings of $42 billion/year [111]. While this expectation appears straightforward, demonstration of such improved outcomes in clinical trials would be a powerful stimulus to bring pharmacogenomics into clinical use in patients with HTN.

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Abbreviations

- MDPI: Multidisciplinary Digital Publishing Institute
- DOAJ: Directory of open access journals
- RAS: renin-angiotensin system
- HTN: Hypertension
- GWAS: genome wide association study
- ACE-Is: angiotensin converting enzyme inhibitors
- ARBs: angiotensin receptor blockers
- MAF: mean allele frequency
- CCBs: calcium channel blockers
- DBP: diastolic blood pressure (DBP)
- DPWG: Dutch pharmacogenomics working Group
- ICAPS: International Consortium for Antihypertensives Pharmacogenomics Studies
- CPIC: Clinical Pharmacogenetics Implementation Consortium

References


