Hypertension genetics past, present and future applications

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Essential hypertension is a complex trait where the underlying aetiology is not completely understood. Left untreated it increases the risk of severe health complications including cardiovascular and renal disease. It is almost 15 years since the first genome-wide association study for hypertension, and after a slow start there are now over 1000 blood pressure (BP) loci explaining ~6% of the single nucleotide polymorphism-based heritability. Success in discovery of hypertension genes has provided new pathological insights and drug discovery opportunities and translated to the development of BP genetic risk scores (GRSs), facilitating population disease risk stratification. Comparing highest and lowest risk groups shows differences of 12.9 mm Hg in systolic-BP with significant differences in risk of hypertension, stroke, cardiovascular disease and myocardial infarction. GRSs are also being trialled in antihypertensive-drug responses. Drug targets identified include *NPR1*, for which an agonist drug is currently in clinical trials. Identification of variants at the *PHACTR1* locus provided insights into regulation of *EDN1* in the endothelin pathway, which is aiding the development of endothelin receptor EDNRA antagonists. Drug repurposing opportunities, including *SLC5A1* and canagliflozin (a type-2 diabetes drug), are also being identified. In this review, we present key studies from the past, highlight current avenues of research and look to the future focusing on gene discovery, epigenetics, gene-environment interactions, GRSs and drug discovery. We evaluate limitations affecting BP genetics, including ancestry bias and discuss streamlining of drug target discovery and applications for treating and preventing hypertension, which will contribute to tailored precision medicine for patients.

Keywords: drug targets, epigenetics, essential hypertension, gene x environment, genetic risk score, genome-wide association study

INTRODUCTION

Hypertension is the leading global risk factor for morbidity and mortality, and studies have demonstrated a clear link between elevated systolic and diastolic blood pressure (BP) and cardiovascular disease (CVD) [1]. In 2015, around 7.8 million deaths were attributed to hypertension [2], and the number of individuals diagnosed with hypertension is estimated to reach 1.5 billion globally by 2025 [3]. The current guidelines proposed by the European Society of Cardiology characterise grade 1 hypertension clinically as ≥140/90 mm Hg in patients below 80 years [4], while the clinical threshold defining hypertension in the United States is lower at ≥130/80 mm Hg, as stated by the American Heart Association [5]. Approximately 95% of hypertensive cases are termed as essential hypertension (EH); hypertension with an unknown cause resulting from interplay of environmental and genetic factors. The remaining 5% of cases are grouped as secondary hypertension, of which 1% are monogenic disorders [6]. Despite extensive research, BP regulation mechanisms and hypertension pathophysiology remain poorly understood, and there are issues in varied patient response and adherence to current pharmacological therapies. For decades, genomic

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research into hypertension and BP has provided clues into its complex genetic architecture in a bid to identify novel target mechanisms for therapeutics and personalised medicines.

HISTORY OF BP GENETICS, EARLY STUDIES AND RESULTS

In 1949, Page documented the multifaceted nature of EH as a result of the dysregulation of four integral systems: cardiovascular, renal, endocrine and neural [7]. The genetic contribution to hypertension was recognised 32 years later in his revision of the Mosaic Theory of Hypertension [8], following evidence from a multitude of familial studies [9–11] and the characterisation of rare monogenic disorders of hypertension [12,13]. With a consensus on the existence of a genetic component of BP, the Platt v. Pickering debate of the 1950s considered whether hypertension was a monogenic or polygenic disorder [14]. Platt argued that rare monogenic disorders of hypertension were evidence for a monogenic nature. In contrast, Pickering postulated the Gaussian, rather than bimodal, distribution of BP throughout the population suggested BP is determined by a collection of genes and further recognised hypertension as a quantitative trait with a normal distribution, opposing the previous suggestion of hypertension as a qualitative trait distinct to normotensive BP [14]. Later studies supported the polygenic nature of hypertension and estimated the heritability of clinical systolic BP (SBP) and diastolic BP (DBP) to be 15%–40% and 15%–30% respectively [9], with rare monogenic disorders representing an extreme end of the distribution.

The Human Genome Project provided the catalyst for advances in gene mapping in the 1990s [15]. Linkage analysis was a key tool in the early years, where microsatellite genetic markers were tested for co-segregation with a trait in families, the results of these studies provided chromosomal locations of genes for traits [6]. Investigation into families with monogenic disorders of hypertension using linkage analysis facilitated the identification of the first BP-associated genes and highlighted the role of renal and adrenal pathways in BP control, as covered extensively by Raina et al [12]. Lifton and colleagues were responsible for the majority of research into monogenic disorders of hypertension in the 1990s - of note, research on patients with Liddle syndrome identified gain-of-function mutations in *SCNN1B* and *SCNN1G* genes encoding subunits of the epithelial sodium channel (ENaC) present on collecting ducts of kidneys,

establishing the role of renal sodium reabsorption in BP control [13]. Yet monogenic forms of hypertension account for a very small percentage of hypertensive cases, and in a bid to elucidate genes involved in polygenic forms of hypertension a series of candidate-gene linkage studies were carried out in familial cohorts [16–22]. These studies yielded some promising results; however, these were often contradictory across cohorts with no single candidate gene consistently showing strong linkage with hypertension. Notably, variants of the *AGT* gene encoding angiotensinogen, a key player in the renin-angiotensin system of BP control, were linked to hypertension in a linkage-analysis of 63 white European families [19]; this result was not replicated in a larger European study of 350 families [20]. Linkage at this locus was subsequently demonstrated across populations, in African-Caribbean [18], Mexican-American [17] and Japanese [21] cohorts, albeit a different variant, but not in others [16], highlighting there may be potential variation between ethnicities. Candidate-gene studies were generally underpowered as they relied on familial cohorts for which recruitment is difficult, and there was a lack replication data, especially from non-white European populations [23]. Furthermore, candidate genes were selected based on previously characterised BP pathways restricting the identification of novel BP genes. At the turn of the 21st century, genome-wide linkage analyses were deployed with the aim of identifying loci anywhere in the genome, a hypothesis free approach. Various genome-wide linkage analyses were undertaken in relatively large cohorts including the Framingham Heart Study [24], the Family BP Program [25,26] and the British Genetics of Hypertension (BRIGHT) study [27]. These studies successfully identified a number of quantitative trait loci (QTLs) (regions of DNA linked to variations in the phenotype) associated with hypertension, some of which were validated in follow-up studies [28–30]. The identification of broad QTL regions on five chromosomes led the BRIGHT study to propose hypertension as an 'oligogenic' disorder, in which a small number of genes located in these regions provide the largest effect on the trait, with additional genes exerting smaller effects [27]. Nonetheless, interpreting linkage analysis results presented challenges and limitations; the QTLs identified spanned broad regions of DNA making the identification of the responsible gene difficult [6], and there was a lack of power to identify variants with smaller effects [31].

Alongside studies in human populations, rodent models have also been studied, and these have provided a valuable resource for understanding the polygenic nature of EH. Importantly, genetically modified mouse models have been instrumental for functional analysis and validation of BP candidate genes [32]. The inbred spontaneous hypertensive rat (SHR) strains have been effective for identifying novel genes and also providing useful physiological models of CVD, as well as a valuable tool for analysis of therapeutic efficacy and toxicity of candidate drugs [33]. The SHR family is composed of several lines of selectively inbred rats, each carrying a different genotype and expressing combinations of traits observed in human EH, as a whole mimicking the human clinical phenotype [33–37]. Prior to the complete sequencing of the rat genome [32], linkage analysis in these rat strains identified over 270 QTL regions associated with hypertensive traits, predominantly located on four chromosomes [34], in line with the oligogenic theory of hypertension [27]. Studies in the rat have some benefits over human studies as the genetic heterogeneity of rat strains, and their environment are easily controllable, increasing study power. Nonetheless, similar to linkage analysis in humans, these studies were limited by their sample sizes, and mapping the QTL regions to identify candidate genes remained a difficult task. Concurrent sequencing of the rat [38] and human [15] genomes provided an opportunity for comparative mapping between species, facilitating more accurate translation of QTL regions from rat studies to humans [33]. We now know that some QTL regions identified with linkage analysis in humans validate in congenic rat strains, this is a topic covered extensively by Padmanabhan and Joe in 2017 [39]. Notably, a meta-analysis of microarray data from SHR identified several genes associated with BP in the rat [40]. A number of the identified genes had previously been associated with hypertension in humans via linkage-analysis or genome-wide scanning, including *APOE* [41], *NPPA* and *NPPB* [42], corroborating the role of these genes in BP control.

THE GWAS ERA – COMMON AND RARE VARIANT DISCOVERY

The complete sequencing of the human genome in 2003 [15], paired with the development of single nucleotide polymorphism (SNP) chip arrays enabled cost-efficient high-throughput genotyping of selected variants and powered the first genome wide association studies (GWAS) [43–48]. SNPs are single base variations in the genome which occur at different frequencies in the population. In GWAS SNPs distributed across the genome are tested for association with traits or diseases. GWASs have benefits over linkage analysis and candidate gene studies as they are unbiased, permit larger sample sizes and enable meta-analyses improving statistical power [49]. The first GWASs for hypertension yielded disappointing results, and no significant loci were found [43,48]. A year later in 2008, the first locus (*ATP2B1*) significantly associated with BP was identified in a GWAS of 1484 Japanese individuals [50]. This result was replicated in a Korean cohort of >8000 individuals [51] and in a large European ancestry cohort of nearly 30,000 individuals [45]. Over the following years, many investigators using GWAS have identified loci for the quantitative traits of systolic, diastolic and pulse pressure [52], facilitating the discovery of novel BP pathways.

SNP arrays include only a small proportion of the variants present in the genome. The development of SNP reference panels, including the 1000 Genomes Project [53] and the Haplotype Reference Consortium [54], enabled imputation and estimation of the effects of associated SNPs not featured on arrays. In tandem with SNP arrays, the establishment of accessible large-scale Biobanks (e.g., UK Biobank) containing not only genetic data but a variety of phenotypic and health-related data [55] has improved the statistical power of GWAS and enabled the detection of both common and rare BP variants [41,56–58]. The first GWASs were powered for the detection of common variants; these have relatively small effect sizes on BP ranging 0.5–1 mm Hg per allele. With increasing access to samples, imputation and sequencing data, investigators have also focused efforts on identifying low frequency and rare variants (minor allele frequency < 1%) which have larger effect sizes (around 1.5 mm Hg per allele). In 2011 the Exome chip was launched [59]; an array of predominantly rare and low frequency variants mostly located in exonic (coding) regions. Four years later, a trans-ethnic meta-analysis of Exome chip data with replication in European and South Asian ancestries identified the first rare exonic variants associated with BP traits with effect sizes greater than that observed with common variants (>1.5 mm Hg per allele), mapped to four genes: *RBM47, COL21A1, DBH* and *RRAS*. In the same year, an additional trans-ethnic study reported a rare variant of the *NPR1* gene, associated with a +1.1 mm Hg increase in SBP; this gene is a drug target for hypertension with a

clinical trial ongoing (Figure 1) [57]. Nonetheless, the collective number of loci identified at this point accounted for 2.8% of the genetic heritability of BP. It was not until 2018 that the impact of large-scale Biobanks was truly demonstrated, with the identification of over 500 novel loci in a single study of one million individuals of European descent, doubling the total explained heritability from less than 3% to ~6% [58]. More recently in 2020, a study including 1.3 million individuals using Exome chip data with replication in trans-ethnic individuals identified an additional 106 novel loci, of which 87 were rare variants [56]. To date, over 1000 loci have been significantly associated with BP, with continuous efforts being made to further unravel the genetic architecture of BP. However, elucidating the causal SNPs remains a challenge; the majority map to noncoding regions of the genome, and variants are often in linkage disequilibrium (LD) with one or more other variants, in which they are non-randomly associated in the population.

GWASs BEYOND EUROPEAN ANCESTRY

Whilst BP genetic discovery projects continue to expand in size, they have maintained a strong bias for centring on individuals of European ancestry, and thus there is limited representation and results from other ancestral backgrounds. This is in part due to recent studies including samples from the UK Biobank which is largely European [55], with cumulative estimates of Europeans contributing 88.45% genotypes to GWASs across all traits in 2020 [60]. In order to have GWAS results that are impactful across populations, this data gap needs to be addressed. Continuing this ancestral bias in genetics could exacerbate health disparities due to ethnicity and miss the benefits of new discovery opportunities and understanding in an inclusive system. African ancestry individuals have the highest age-adjusted prevalence of hypertension [61]. Downstream issues are already occurring due to ancestral bias; using genetics for risk stratification of cardiomyopathy wrongly miscategorises some African Americans due to their omission from control cohorts [62]. This demonstrates the importance of interrogating the population-specificity of identified variants. Research across ancestries is increasing, either through sampling less studied populations or conducting trans-ethnic studies [41,63]. These studies have discovered novel and ancestry specific loci, although they are often limited by smaller sample sizes in com-

parison to European-based research [64]. An increase in diverse sampling is being addressed by ongoing establishment of national biobanks (H3Africa, BioBank Japan, the Korean Biobank, the African Genome Variation Project, Qatar Biobank and GenomeAsia 100k), and these datasets are being increasingly utilised in genomics research [65,66].

RISK PREDICTION AND CAUSAL MECHANISMS

As BP GWAS summary statistics and other datasets become publicly available to researchers (ebi.ac.uk/gwas/, genetics.opentargets.org/, phenoscanner.medschl.cam.ac.uk/), new methods for interpreting and translating these data have been developed for clinical applications and biological interpretation of findings.

Risk prediction modelling for CVD is now including genetic biomarkers. Genetic risk scores (GRSs) can be developed from combining significant risk alleles identified from GWAS. Alternatively, a more complex polygenic risk score (PRS) can be created by combining a broader range of SNPs which may not individually reach genome-wide significance but together provide an improved risk score [67]. One recent BP-GRS developed in UK Biobank (n = 392,092) combining 901 SNPs found a difference of 12.9 mm Hg (SBP) and 7.5 mm Hg (DBP) between the lowest risk decile and the highest risk decile along with a trebled risk of hypertension and an increased risk of stroke, CVD and myocardial infarction [58]. The authors of this study indicated such a risk score may have utility for early identification of individuals, at a time where lifestyle factors could be advocated to reduce BP levels. PRSs have also been used to test for responsiveness to different drug classes. One such recent study evaluated the association of a genome-wide PRS (>1 million SNPs) with antihypertensive drug responses. This was conducted across four BP drug classes based on data with a sample of $n{\sim}200$ per drug using BP data before and after 4 weeks on monotherapy; however no association was established [68]. Utilising risk scores in this way is an area of research in its infancy, and this is reflected in the small sample sizes studied and consequently lower power which could influence the results obtained so far. Increased sample sizes would be important to account for any BP measurement variance. Furthermore, the studies in this area are predominantly conducted in individuals from European ancestries, potentially leading to
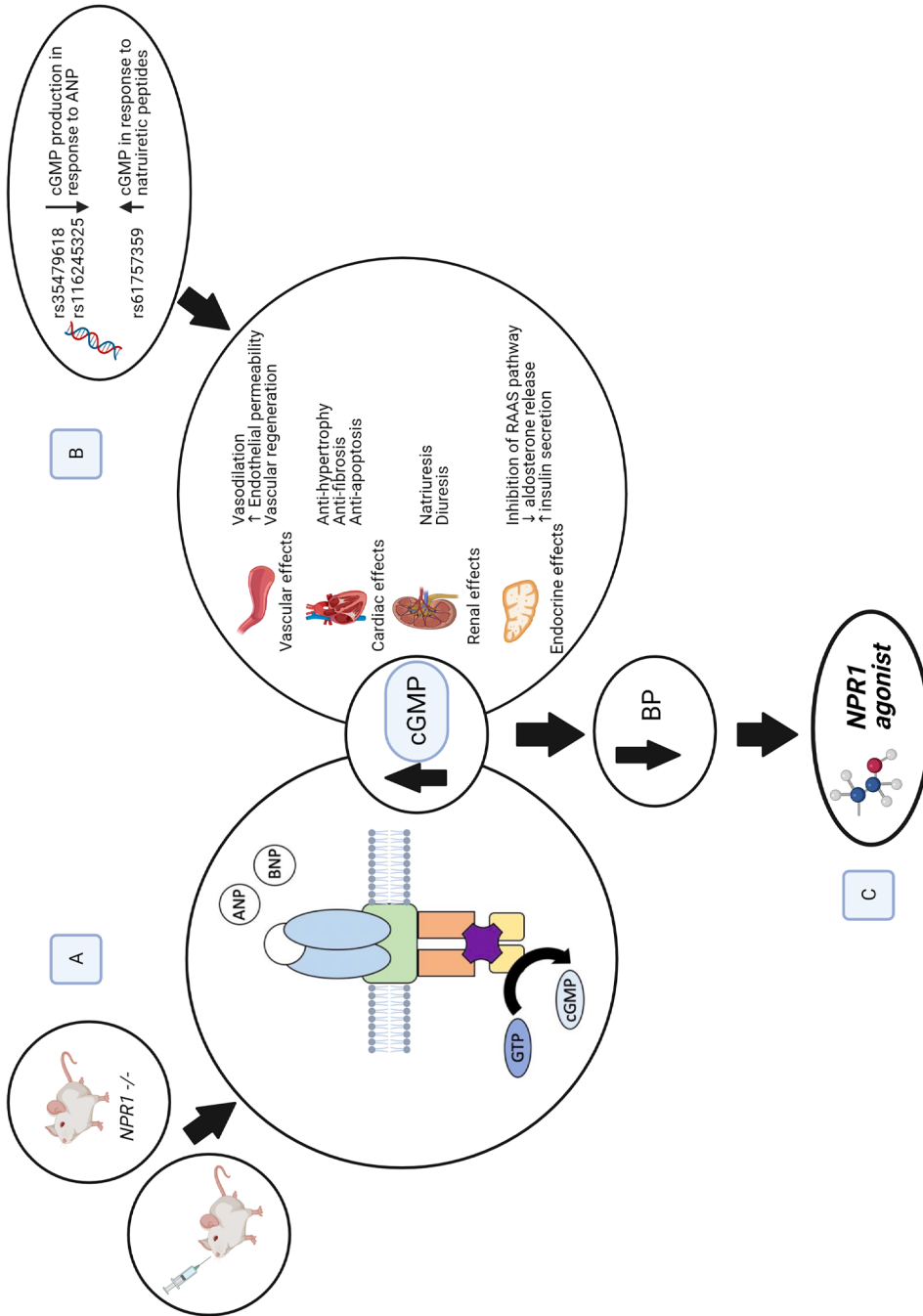
FIGURE 1 *Natriuretic peptide receptor 1: Discovery, genetics and development as a therapeutic target. The NPR1 gene encodes the natriuretic peptide receptor 1, atrial and brain natriuretic peptides (ANP/BNP) bind to this receptor, their binding leads to lower BP and salt extraction [105]. (a) This mechanism was first identified by injecting atrial extracts into rats resulting in natriuresis [106]. This responsible factor was soon identified, sequenced and labelled as ANP [107]. Further discoveries led to discovery of ANP binding receptors (NPR-A) which regulate cGMP with npr1 knockout mice models and those with gene-targeting establishing a dose-response effect [107, 108]. NPR1 encodes the NPR-A transmembrane protein. Binding of ANP or BNP results in the conversion of GTP to cGMP which then activates various other proteins (cGMP-dependent protein kinase I and II, PDEs and CNGs) resulting in the eventual downstream effects of lowered BP [105]. (b) In 2016 rare variants in NPR1 were identified as associated with blood pressure in an exome analysis [57]. The genetic variants mapped to specific alterations in protein structure, and subsequently effects were determined through in vitro experiments. Allelic changes in SNPs rs35479618 and rs116245325 result in differences in the catalytic guanylate cyclase domain which are associated with increased BP as ligand binding results in decreased cGMP production. Changes at SNP rs61757359 result in alterations to the protein kinase-like homology domain increasing cGMP production in response to receptor activation and are consequently associated with decreased BP [109]. (C) A NPR1 agonist drug (REGN5381) is in development. This is being evaluated in a Phase I clinical trial for its use as a hypertension treatment (NCT04506645) with initial results expected in the summer of 2021*

Abbreviations: ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; BP, blood pressure; CNG, cyclic nucleotide gated channel; GTP, guanosine triphosphate; cGMP, cyclic guanosine monophosphate; PDE, phosphodiesterase; RAAS, renin-angiotensin-aldosterone system; SNP, single nucleotide poly-morphism.

effectiveness issues across ethnicities due to the intrinsic limitations of the PRSs [69].

Alongside the development of risk scores, Mendelian randomization (MR) is being applied widely to determine causal effects using genetic data to mimic a randomized controlled trial [70]. The MR framework uses SNPs to overcome issues with traditional observational studies such as bias due to confounders and reverse causation. As a random assortment of alleles is passed onto offspring independent of any other characteristics, SNPs in a given population should be similar in all other characteristics removing any potential confounders [70]. Since inception, MR has been regularly applied to genetic data to identify causal risk factors, drug evaluation and identifying disease mechanisms. A recent MR study used a novel approach to assess whether repurposing antihypertensive drugs would affect the risk of Alzheimer's disease [71]. The study was conducted using SNPs in genes associated with 12 antihypertensive drug classes. The selection of SNPs was based on data from www.drugbank.ca/ and www.gtexportal.org/, and these were validated in a UK Biobank SBP GWAS cohort ($n = 317,754$), and the effect was estimated in an Alzheimer's disease GWAS ($n = 17,008/37,154$ case/control). The results indicated lowering SBP via the antihypertensive drug targets selected was unlikely to affect the risk of developing Alzheimer's disease. This work provides a blueprint for evaluating antihypertensive drug applications without conducting a full randomized controlled trial [71]. In another example, Richardson and colleagues used the principles of MR to study the association of the transcriptome across 48 tissue types with complex traits to identify candidate genes loci [72]. Their analysis applied to BP data identified possible causal associations, for example one SNP (rs1706003) which may have been overlooked by using GWAS data alone indicated the candidate gene *ATP13A3* [72]. These methods serve as a reminder that a variety of approaches are available and are being constantly developed with numerous applications to utilise and interpret results from genetic studies.

Determining candidate genes and mechanisms at BP loci is key for translation to druggable targets. Before the advent of GWAS, genes and mechanisms for BP were mostly discovered using rat or mouse models. Now, GWAS is taking centre stage, and combining their results with experimental models

provides additional support for drug development as illustrated by *NPR1* (Figure 1).

As mentioned previously, GWASs do not provide the causal variant or gene. Functional studies using mouse models remain a key experimental tool once a gene is identified as having strong support from bioinformatics analysis. A recent example of follow-up of GWAS loci is demonstrated with the identification of *ARHGAP42* (Rho GTPase Activating Protein 42) as the result of the SNP rs633185 reported as a lead variant at this locus in a GWAS [73]. Genetically modified mice were used to establish that ARHGAP42 deficiency results in hypertension via increased response to angiotensin II and endothelin-1 [74]. These models are continuing to be applied to assess whether this candidate gene could be a valid drug target moving forward [75]. Nonetheless, functional validation represents a major challenge due to the large number of SNPs associated with BP. Candidate genes from GWAS can be evaluated using 'in vitro' systems, including techniques such as CRISPR which can be used for gene-editing of BP variants and subsequent testing in cellular models. Alternatively, mechanisms for GWAS 'candidate genes' can converge from others work studying known BP mechanisms. For example, the variant rs880315 located within an intron of *CASZ1* (Castor Zinc Finger 1) is associated with hypertension in GWASs and replicates across different populations and ancestries [45,76,77]. A recent study has established that CASZ1b (short form of *CASZ1*) co-localises with the mineralocorticoid receptor in the kidneys and is part of an aldosterone-dependent corepressor complex suppressing ENaCα and SGK1 which are linked to elevating BP by promoting sodium reabsorption [78].

GENETICS PRIMING DRUG DISCOVERY

Developing new drug treatments for EH is a key driver of BP genetics research. Increasing our armoury of therapies that effectively lower BP with minimal side effects and reduce hypertension-associated CVD is important for personalised medicine. This is particularly necessary for hypertension as there are a large proportion of individuals that do not respond to current treatments. Evangelou et al reported five loci (*PKD2L1, SLC12A2, CACNA1C, CACNB4* and *CA7*) containing genes which are drug targets for several known antihypertensive drug classes [58]. These genes strongly validate the genetic approach of identifying potential drug targets. With greater than

1000 BP-associated loci now identified and genetic data being collected each year, the list of possible drug-target genes is continuously expanding. For example, in 2020 Surendran et al reported 23 genes as potentially druggable [56]. However, only 12 of the potentially druggable genes identified by Evangelou et al are the focus of clinical trials for BP (Table 1), including the gene *EDNRA*. *EDNRA* encodes endothelin receptor A which plays a role in the endothelin pathway, an established mechanism of BP control (Figure 2). Although, *EDNRA* can be considered as a drug-target this is mentioned with caution, as currently endothelin receptor antagonists that are used clinically to treat pulmonary hypertension have had road-blocks when reaching clinical applications for EH [79]. There are also druggable genes not identified initially via GWAS but from other genetic studies which are in development, including the gene *MTHFR* (Figure 3). Work is in progress to validate the mechanism by which *MTHFR* potentially impacts BP. It is hoped that some of these discoveries will translate to novel therapeutics.

Alongside identifying new therapeutic targets, genetics also provides insight into opportunities for drug repurposing. Evangelou et al and Giri et al both curated lists of potential gene-drug interactions and identified genes with drug-targets with repositioning potential for BP (e.g., *MARK3, PDGFC, TRHR, ADORA1, GABRA2, VEGFA, PDE3A* and *SLC5A1* noted by Evangelou et al and *PDE3A, PSMB9* and *SH2B3* noted by Giri et al) [41,58]. One of the strongest contenders was *SLC5A1*, the target of canagliflozin. Canagliflozin is a SGLT2 inhibitor and originated as a therapeutic for type-2 diabetes. It has since been licensed for treatment in heart failure. Canagliflozin decreases glucose reabsorption but also reduces BP in diabetes patients, indicating potential as an anti-hypertensive therapeutic [58].

Pathway enrichment analysis is a post-GWAS test which can provide mechanistic insights and highlight organ systems and signalling pathways which could be therapeutically targeted. Notably from BP GWASs there is enrichment of genes in arteries, and TGF-ß and Notch signalling pathways are indicated [41,56]. However, a lack of enrichment in other tissues, including the kidneys which are heavily involved in BP regulation, highlights caveats in tissue enrichment analysis (noting there are limited kidney samples in public datasets) [41]. Nevertheless, pathway analyses have successfully reported genes associated with BP and other CVD pathophysiologies, including *PHACTR1*, informing possible interplay between disease mechanisms [41].

RESISTANT HYPERTENSION

Resistant hypertension, defined as high BP despite patients being on three antihypertensive drug classes [80], is not as well studied using genetic approaches. This is in part due to the fact that resistant hypertension is a complex clinical entity, in which resistant hypertension is also used as an umbrella term for any elevated BP that is nonresponsive where you can rule out nonadherence and secondary causes. This phenotype can result from a large range of diverse pathophysiologic sources and thus poses a great challenge for reliably identifying significant findings in genetic studies. Furthermore, BP GWASs have focused heavily on quantitative BP phenotypes as described therein; however in recent years GWASs for resistant hypertension have been performed [81,82], with some new findings. For example, Rouby et al performed, to the best of our knowledge, one of the first GWAS for resistant hypertension and used their findings to develop a GRS [81]. The GRS was based on three signals (in *MSX2, IFLTD1* and *PTPRD*) that were found in participants taken from two randomized clinical trials (1194 White and Hispanic participants in the discovery stage from one clinical study and 585 individuals in the replication stage from an independent clinical study) [81]. The GRS is yet to be tested with researchers noting there was no cohort large enough at that time to study [81]. In contrast, Irvin et al performed a resistant hypertension GWAS, in which they replicated results in new samples using the Million Veterans Program ($n = 16,833$) with replication across different ethnic groups and identified the *CASZ1* locus with greatest significance [82]. Specifically, they found that rs12046278 T carriers in *CASZ1*, a locus also associated with quantitative BP traits, were less likely to have resistant hypertension [82]. Overall, these findings emphasize resistant hypertension GWAS is in its infancy and requires further research. New findings have the potential to improve our understanding of resistant hypertension BP biology and to optimize how BP drugs are prescribed.

BP GENETICS BEYOND GWAS - EPIGENETICS

Whilst GWAS has enabled the discovery of a large number of loci, they only explain approximately 6% of BP SNP heritability thus far [58]. Epige-

TABLE 1. Genes identified at blood pressure loci by Evangelou et al. [58] which have drugs in clinical trials relating to hypertension. Gene symbols were identified within downloaded description data for all clinical trials searched for under the term 'blood pressure' within clinicaltrials.gov (9502 clinical studies. Genes not mentioned by their HGNC gene symbols were not captured in the automated table curation

Gene	Gene description	Interacting drug mechanisms (GeneCard)	Pathways (KEGG and reactome)	Diseases (OMIM)	NCT numbers (clinical studies for 'blood pressure')
ACE	Angiotensin I converting enzyme	ACE inhibitor, vasoconstrictor, selective miner-alocorticoid receptor antagonist, NKCC cotransporter inhibitor, Na+/2Cl-/K+ (NKCC) symporter inhibitor	hsa04020:Calcium signaling pathway, hsa04022:cGMP-PKG signaling pathway, hsa04024:cAMP signaling pathway, hsa04080:Neuroactive ligand–receptor interaction, hsa04261:Adrenergic signaling in cardiomyocytes, hsa04540:Gap junction, hsa04923:Regulation of lipolysis in adipocytes, hsa04924:Renin secretion, hsa04970:Salivary secretion, hsa05414:Dilated cardiomyopathy	607276:Resting heart rate, Congestive heart failure and beta-blocker response	NCT02063477, NCT01403922, NCT00185120, NCT00932867, NCT03508895, NCT01963507, NCT03587103, NCT02214498, NCT02184858, NCT01234922, NCT00666848, NCT02130687, NCT01152567, NCT01444833, NCT03295734, NCT02517866, NCT01454583, NCT00985322, NCT00374270, NCT00994253, NCT01071642, NCT02059408, NCT00980187, NCT02807987, NCT00126516, NCT04331574, NCT01891513, NCT00980785, NCT04330300, NCT00157729, NCT04026776, NCT01413542, NCT01217879, NCT01211171, NCT00210262, NCT04334824, NCT00221845, NCT03121092, NCT00308386, NCT00118976, NCT01586897, NCT01380717, NCT00051389, NCT00005757, NCT00399737, NCT01832558, NCT01705392, NCT01118520, NCT02991534, NCT01243138, NCT00241904, NCT00000542, NCT02845063
ADRB1	Adrenoceptor beta 1	βadrenergic receptor antagonist	hsa04614:Renin-angiotensin system, hsa04924:Renin secretion, hsa05142:Chagas disease (American trypanosomiasis), hsa05410:Hypertrophic cardiomyopathy (HCM)	104300:Alzheimer disease, 267430:Renal tubular dysgenesis, 612624:Microvascular complications of diabetes 3, 614519:Stroke	NCT02398929

(Continued)

TABLE 1. Continued

Gene	Gene description	Interacting drug mechanisms (GeneCard)	Pathways (KEGG and reactome)	Diseases (OMIM)	NCT numbers (clinical studies for 'blood pressure')
AGT	Angiotensinogen	ACE inhibitor, calcium channel blocker, angiotensin II inhibitor	hsa04614:Renin-angiotensin system, hsa04924:Renin secretion	145500:Essential hypertension 267430:Renal tubular dysgenesis	NCT03714776, NCT03934307, NCT04083222, NCT02796170
ARNTL	Aryl hydrocarbon receptor nuclear translocator like	NA	hsa04710:Circadian rhythm, hsa04728:Dopaminergic synapse, hsa05168:Herpes simplex infection	NA	NCT02249793
CLCNKA	Chloride voltage-gated channel Ka	Ca2+-activated Cl-channel blocker, Cyclooxygenase-2 (COX-2) inhibitor, GPR35 agonist	R-hsa-2672351:Stimuli-sensing channels	613090:Bartter syndrome	NCT01275352
EDNRA	Endothelin receptor type A	Endothelin-A and Endothelin-B receptor antagonist	hsa04020:Calcium signaling pathway, hsa04022:cGMP-PKG signaling pathway, hsa04024:cAMP signaling pathway, hsa04080:Neuroactive ligand–receptor interaction, hsa04270:Vascular smooth muscle contraction, hsa04924:Renin secretion, hsa05200:Pathways in cancer,	157300:Migraine, 616367:Mandibulofacial dysostosis with alopecia	NCT03038750

(Continued)

TABLE 1. *Continued*

Gene	Gene description	Interacting drug mechanisms (GeneCard)	Pathways (KEGG and reactome)	Diseases (OMIM)	NCT numbers (clinical studies for 'blood pressure')
PROCR	Protein C receptor	NA	R-hsa-140875:Common Pathway of Fibrin Clot Formation	614224:Retinal arterial macroaneurysm with supravalvular pulmonic stenosis	NCT03038750
IGFBP7	Insulin-like growth factor binding protein 7	NA	hsa04060:Cytokine-cytokine receptor interaction, hsa04066:HIF-1 signaling pathway, hsa04068:FoxO signaling pathway, hsa04151:PI3K-Akt signaling pathway, hsa04620:Toll-like receptor signaling pathway, hsa04621:NOD-like receptor signaling pathway, hsa04623:Cytosolic DNA-sensing pathway, hsa04630:Jak-STAT signaling pathway, hsa04640:Hematopoietic cell lineage, hsa04668:TNF signaling pathway, hsa05410:Hypertrophic cardiomyopathy (HCM)	108010: Intracranial hemorrhage in brain cerebrovascular malformations, 125853:Diabetes, 222100, 148000:Kaposi sarcoma, 266600:Crohn disease-associated growth failure, 604302:Rheumatoid arthritis	NCT03431181

(Continued)

TABLE 1. *Continued*

Gene	Gene description	Interacting drug mechanisms (GeneCard)	Pathways (KEGG and reactome)	Diseases (OMIM)	NCT numbers (clinical studies for 'blood pressure')
IL6	Interleukin 6	NF-κB signaling Inhibitor, HMG-CoA reductase inhibitor, PPARalpha agonist, P38α MAPK inhibitor, highly selective and ATP-competitive, Antioxidant;mucolytic agent	hsa00670:One carbon pool by folate, hsa01100:Metabolic pathways, hsa01200:Carbon metabolism	181500: Schizophrenia, 188050:Thromboembolism, 236250:Homocystinuria due to MTHFR deficiency, 601634:Neural tube defects	NCT02421835, NCT00669435, NCT00471341, NCT00976872, NCT00296218, NCT01419912, NCT02044471
MTHFR	Methylenetetrahy reductase	VEGF antagonist, Folate antagonist, RNA processing inhibitor, reversibly stimulates SERCA Ca2+-ATPase	hsa00230:Purine metabolism, hsa04022:cGMP-PKG signaling pathway, hsa04024:cAMP signaling pathway, hsa04270:Vascular smooth muscle contraction, hsa04921:Oxytocin signaling pathway, hsa04923:Regulation of lipolysis in adipocytes, hsa04924:Renin secretion, hsa04925:Aldosterone synthesis and secretion	NA	NCT03151096, NCT02463513, NCT04278378

(Continued)

TABLE 1. *Continued*

Gene	Gene description	Interacting drug mechanisms (GeneCard)	Pathways (KEGG and reactome)	Diseases (OMIM)	NCT numbers (clinical studies for 'blood pressure')
NPR1	Natriuretic peptide receptor 1	Atrial natriuretic peptide receptor A agonist	R-has-578768:Physiological factors	NA	NCT04506645
UMOD	Uromodulin	NA	R-hsa-446203: Asparagine N-linked glycosylation	162000: Hyperuricemic nephropathy, familial juvenile 1, 603860:Medullary cystic kidney disease 2, 609886:Glomerulocystic kidney disease with hyperuricemia and isosthenuria	NCT03354897

Abbreviations: HGNC, HUGO gene nomenclature committee; OMIM, online mendelian inheritance in man.
Database URLs: GeneCard: https://www.genecards.org/, OMIM: https://www.omim.org/, KEGG: https://www.genome.jp/kegg/pathway.html, Reactome: https://reactome.org/, Clinical trials: https://clinicaltrials.gov
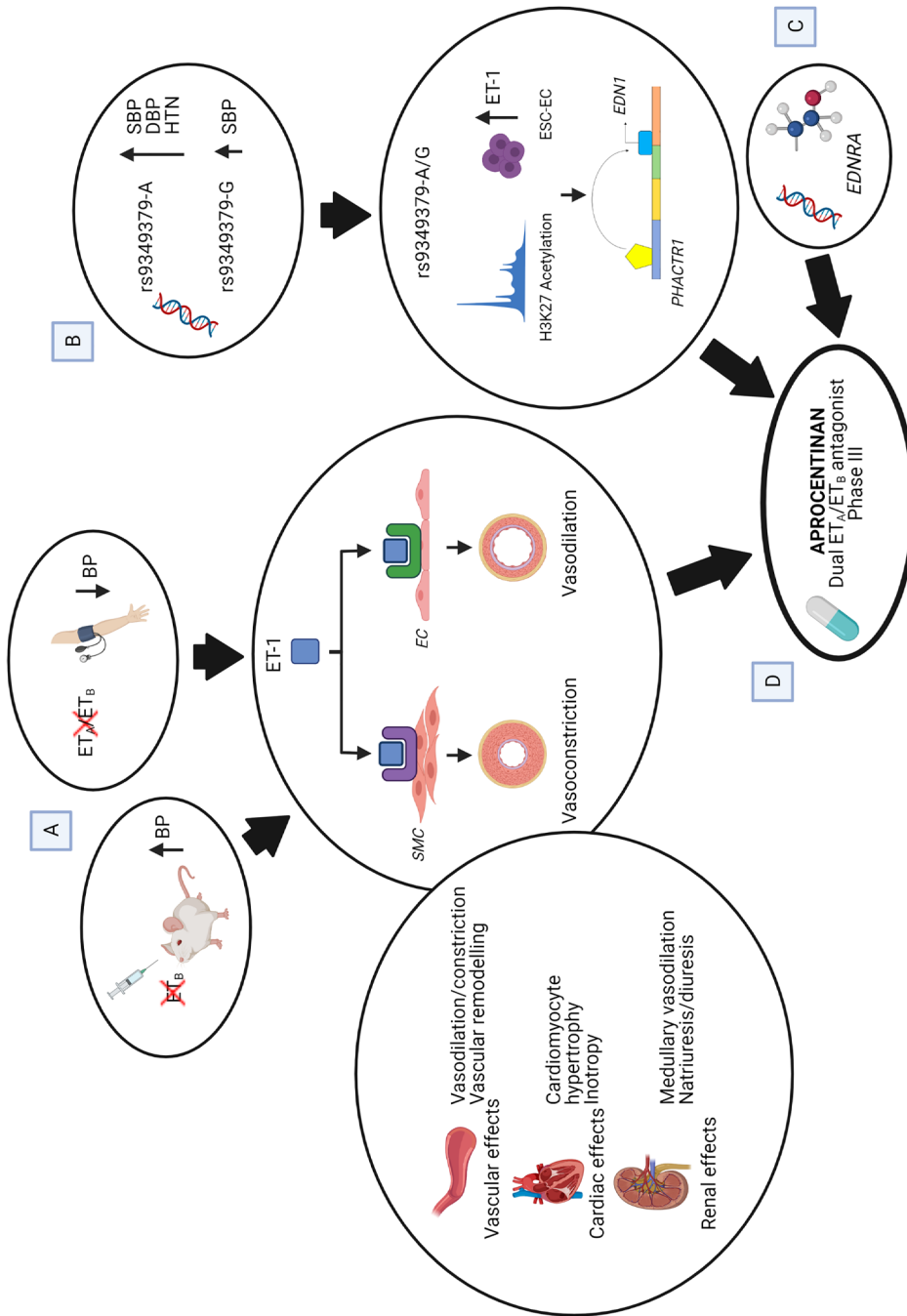
FIGURE 2 *Targeting endothelin receptors for treating essential hypertension. The endothelin-1 (ET-1) pathway has been recognized since the 1980s as playing an important role in BP control [110]. (a) Studies in rodent models [111, 112, 113] and humans [114] have shown that ET$_A$ and ET$_B$ receptors work in tandem to regulate blood vessel contraction, highlighting endothelin receptors as a druggable target for hypertension treatment. (b) Genomic analysis has recently identified the SNP rs9349379, a non-coding variant at the PHACTR1 locus to be associated with elevated systolic and diastolic BP and increased risk of hypertension [41], [115], [116]). Fine-mapping of this locus confirmed rs9349379 as the causal SNP, and H3K27 acetylation analysis in aortic tissues identified this variant as a distal regulatory enhancer [117]. Deletion of 88 base pairs at the PHACTR1 locus in CRISPR-edited stem cell-derived endothelial cells (ESC-EC) resulted in elevated expression of the endothelin-1 (EDN1) gene and ET-1 protein production compared with wild-type cell lines [117]. These data suggested the variant acts as a distal regulator of the EDN1 gene located 600 kb upstream of PHACTR1 and provided further support for targeting the endothelin pathway for hypertension treatment. (c) Analysis of gene drug interactions identified the gene encoding the ET$_A$ receptor (EDNRA) as druggable in hypertension treatment [58]. (d) Previous ET receptor antagonists have failed clinical trials due to adverse effects (Krum et al, 1998) and/ or unexpected failed efficacy [119]. Aprocentinan, a dual ET$_A$/ET$_B$ receptor antagonist developed for hypertension treatment, has shown efficacy in decreasing BP in Phase II clinical trials published in 2020. Phase III clinical trials are currently underway assessing drug safety and efficacy in 600 patients with diagnosed resistant hypertension, in combination with previously prescribed anti-hypertensive drugs, with results expected in early 2022 (ClinicalTrials.gov ID: NCT03541174) Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; EC, endothelial cell; ESC-EC, embryonic stem cell-derived endothelial cells; ET-1, endothelin 1; HTN, hypertension; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; SMC, smooth muscle cells.*
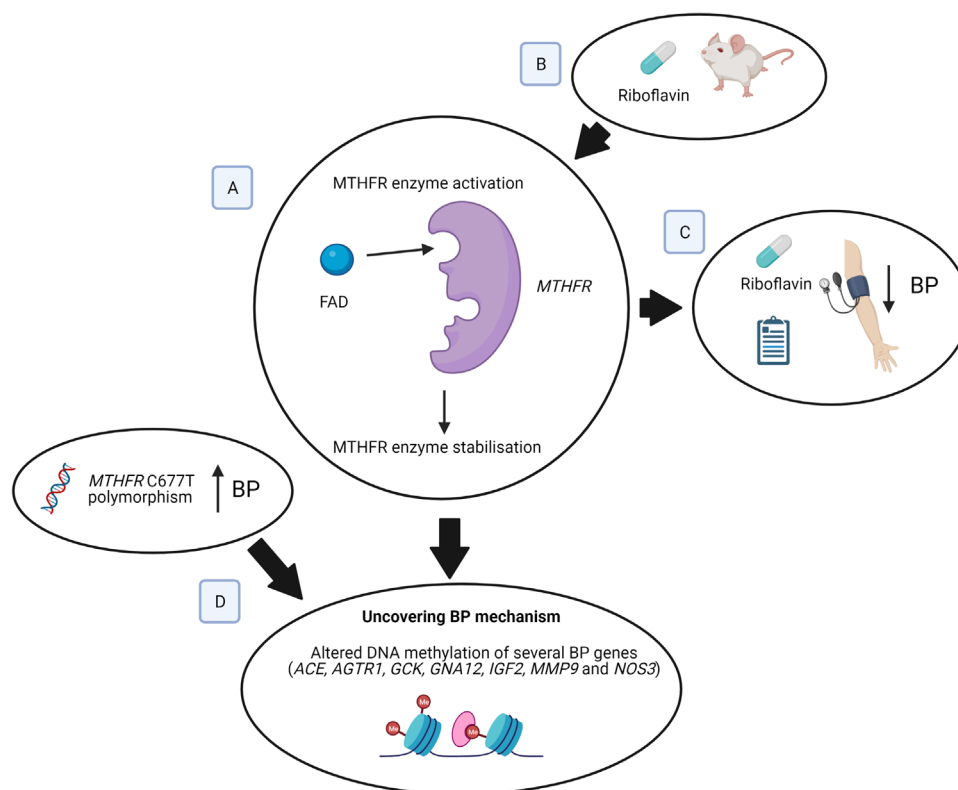
FIGURE 3 *MTHFR: Uncovering novel blood pressure mechanisms.* MTHFR *has been a research topic since 2002 when C677T polymorphism was identified [120], alongside research demonstrating an association of this variant with BP [44]. Its actual BP mechanism however has remained elusive. Studies have shown* MTHFR *has a BP altering relationship with the B vitamin riboflavin. (a) Riboflavin in its co-enzymatic form FAD is required as a cofactor for the MTHFR enzyme and was found to stabilize the MTHFR enzyme in animal models with the* MTHFR *C677T polymorphism [121]. (b) This relationship has since been explored in clinical trials investigating riboflavin supplementation and BP (ClinicalTrials.gov ID: NCT03151096, NCT02463513). (c) Recently, Amenyah et al. [122] found individuals with the* MTHFR *677TT vs CC genotype in response to riboflavin had altered DNA methylation at several blood pressure genes (*NOS3, ACE, GNA12 *and* AGTR1*), suggesting a potential mechanism by which riboflavin is able to lower BP, and indicating methylation affects* MTHFR's *impact, presenting hypotheses for further epigenetic research*
Abbreviations: BP, blood pressure; FAD, flavin adenine dinucleotide; MTHFR, methylenetetrahydrofolate reductase.

netic changes may also have an important role in the heritability of BP and may explain some of the heritability not accounted for by SNP variation. Epigenetic changes (modifications that lead to changes in the expression of genes but do not change the DNA sequence) can be both heritable and modulated through environmental factors, for example nutrition [83]. Epigenetic mechanisms can alter the expression of specific genes through various methods including DNA methylation, which is often found at CpG dinucleotides (cytosine and guanine bases connected via a phosphodiester bond) located in promoters of genes [84]. Some known BP loci have already been shown to act through epigenetic mechanisms (see the *PHACTR1* locus rs9349379 interaction with the endothelin pathway in Figure 2). The development of arrays targeting CpG sites has allowed the investigation of epigenetic BP regulation using EWASs (epigenome-wide association studies). BP EWASs are still in the early stages of exploration compared to GWAS with only a small number of studies published each with limited sample sizes and therefore low power. One of the largest BP EWAS (17,010 individuals of European, African American, and Hispanic ancestry) reports 13 methylation loci which account for an additional 1.4%–2% of heritable BP variation. The results were validated

in a cohort of 1516 individuals, along with 126 loci identified after meta-analysis [85]. Another recent EWAS has suggested potential ethnic differences in methylated sites [86]; however this was based on a significantly smaller sample size ($n = 712$ comprised of South Asian and European ancestries) identifying eight loci (one present in both ethnicities, seven European only). This study did not have a validation cohort; however, it included a comparison between their results and the aforementioned study, which identified some overlap (e.g., cg19693031 near the *TXNIP* gene) with weaker evidence of association compared to the previous study. This in part could be due to both the smaller sample size and the differing ethnic background of this study (49% South Asian descent). Understanding epigenetics and its contribution to hypertension is currently limited by technology. The current array being used in EWASs covers <2% of known CpG sites [87] as well as not detecting the effect of other common epigenetic mechanisms such as histone modification and non-coding RNA which can also be seen work in tandem with each other (for example lncRNA regulating DNA methylation [88]). Further research in this area will be forthcoming and will provide insights into how epigenetics may mediate the relationship between BP genetics, environmental factors and CVD.

GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS

There is a lot of on-going work investigating gene-gene and gene-environment interactions, results of which may advance our understanding of why BP drugs are effective in only some patients and aid the development of targeted drugs regulating gene interactions. To the best of our knowledge, BP gene-gene interactions have only been assessed in a handful of small-scale studies [87,89,90]. For example, Meng et al found five novel interactions (*MAN1A1, LMO3, NPAP1/SNRPN, DNAL4* and *RNA5SP455/KRT8P5*) contributing to hypertension [91]. However, this study only included 2203 cases with a matched number of controls from a predominantly European dataset [91]. The identified genes require further research to definitively prove a link to BP modulation. There are also several other similar studies with small datasets (less than 1000 hypertensive cases per study [87,89,91]). These studies have identified novel interactions between known BP genes (for example between *MTHFR* and *FGF5* [89]). This work emphasizes a need for future work scaling up gene-gene studies across populations for validation - from

which gene expression may be regulated as part of more precise treatment as we gain better understanding of the interplay between genes.

There is more work on gene-environment interactions. The goal of this approach is to understand exactly how environmental factors (e.g., smoking, alcohol intake, air pollution) interact with genotypes to affect BP. These interactions may lead to preventative medicine for at risk individuals depending on modifiable environmental factors. In 2018, Rao et al, established the Gene-Lifestyle Interactions Working Group to develop robust investigation of gene-environment interactions [92]. This working group provided a study design that enables large-scale gene-environment study and consists of 610,475 individuals from 124 cohorts [92], establishing a framework for future study. A recent study by Sung et al included gene-smoking interactions in their multi-stage GWAS, finding 30 loci that were only associated with individuals of African ancestry [93]. Eight of these loci had significant interactions with smoking status, such as the *CSMD1* locus which was also associated with BP in SHRs [93]. However, these loci were only identified in the discovery stage of the study, and, due to the small sample size of the African individuals, replication was not possible [93]. The results from this analysis and other studies [94] further highlight a need for future work to incorporate larger sample sizes of non-European populations.

MINING POST-GWAS DATA

Post-GWAS methodology is advancing to meet growing demands for analysis of large amounts of genetic data. High-powered computational methods can account for SNP associations and their annotations in-depth, guiding hypotheses for functional follow-up, avoiding cherry-picking bias, and dissecting past LD to the causal disease genes. The methods being increasingly deployed range from machine learning, network analysis, fine-mapping, text-mining, MR and hybrid tools (Figure 4). Machine learning is a statistical method using algorithmic rules to identify complex data patterns and make predictions. It has had investment due to its ability to identify hidden patterns within multi-omic data. Mishra et al have used deep learning to identify the functionality of BP SNPs, finding they had a higher likelihood of appearing in CTCF-binding regions [95] – sites that regulate transcription and enable chromatin
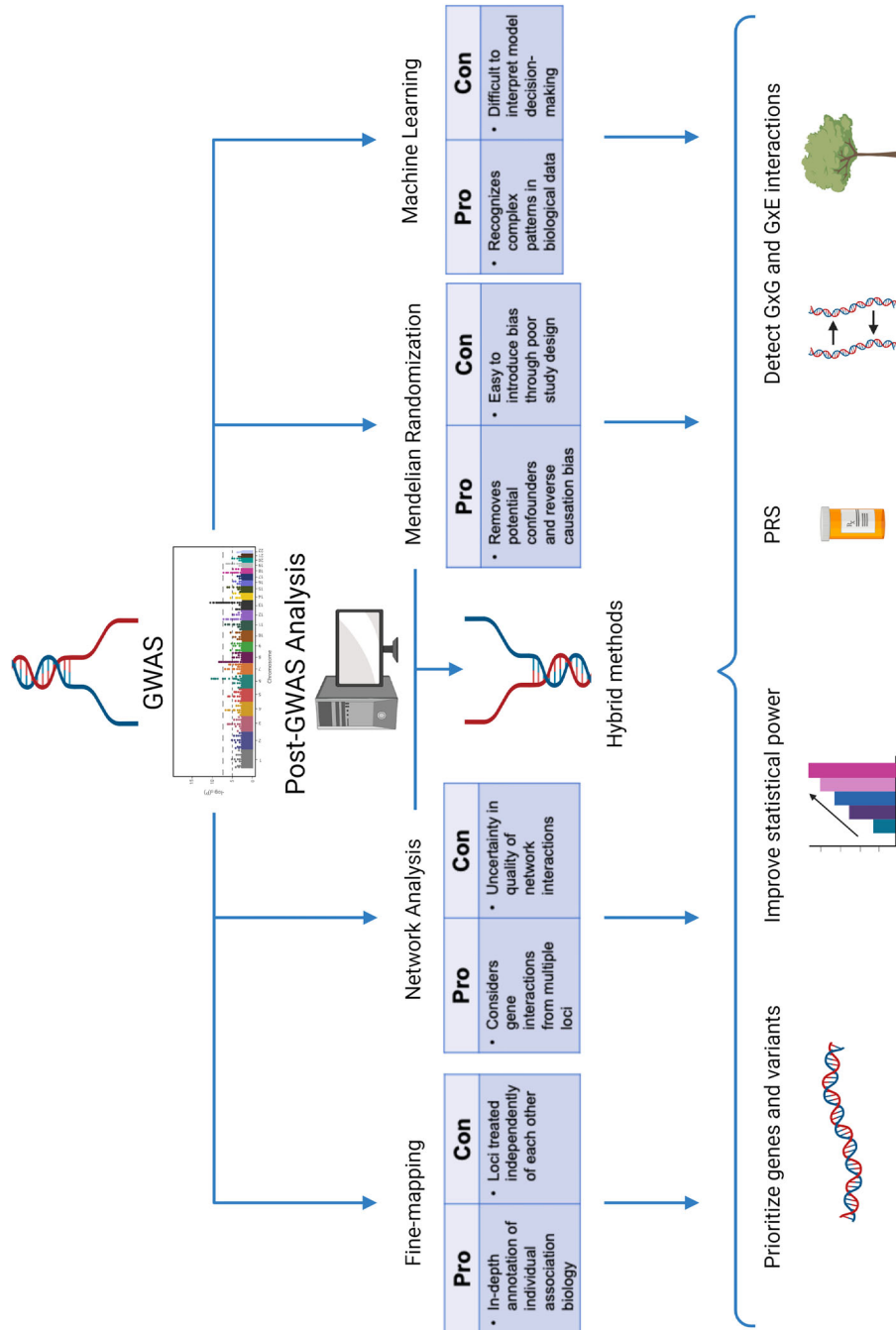
FIGURE 4 *Overview of developing post-GWAS analysis methods.*
Post-GWAS methods are being optimized for several endgame GWAS goals, such as fine-mapping and network analysis for identifying candidate disease genes, Mendelian randomization for PRS, or machine learning for several applications including improving GxG and GxE interaction detection. These approaches combined with recent development of hybrid methods are expected to surpass individual method disadvantages and advance translation from GWAS results
Abbreviations: GWAS, genome-wide association study; GxE, gene-environment interactions; GxG, gene-gene interactions; PRS, polygenic risk score.

loops. This method offers the ability to illuminate details about the majority of BP SNPs found in regulatory regions and provides evidence for laboratory follow-up of select genes [95]. Machine learning has also been used broadly across traits to understand GWAS results (predicting epistatic interactions, PRSs, or prioritizing variants/genes) with potential for translational benefit. Paré et al used machine learning to augment PRSs for polygenic traits, showing machine learning coupled with summary-level GWAS data improved the R^2 prediction of PRSs for height, body mass index and diabetes [96]. These results suggest machine learning may also enhance PRSs for hypertension, indicating its capability not only in understanding the basic science of BP, as shown by Mishra et al, but also as a translational tool. However, novel applications such as this are also limited - for example, machine learning being a 'blackbox' with lack of explainability - indicating an advantage for developing hybrid methods. For example, Hemani et al combined MR with machine learning for causal inference post-GWAS, identifying causal variants across 2407 phenotypes and creating a causality map of the human phenome [97]. As innovative methods such as these develop, their work paves the way for future studies to extract higher level information from amassing BP GWAS results and sequencing data.

CHALLENGES AND OUTLOOK

Genetics research is starting to provide a more comprehensive understanding of how individual pathways and systems contribute to hypertension. However, the specifics of how BP regulatory mechanisms interact with each other (e.g., epigenetic factors acting in known BP pathways, gene-gene, gene-environment, and gene-microbiome interactions) is largely unexplored. A recent review by Weber et al describes the regulation of hydrogen sulfide metabolism and interactions with epigenetic factors and gut microbiota and their contribution to hypertension [98]. From their research they put forward the theory that hydrogen sulfide metabolism can be dysregulated and affect renal function and BP, by gut microbiota (specifically bacterial activity that increases urea blood concentration) and microRNA modulation of angiotensin II [98]. This study integrated results from multiple modalities - epigenetics, metabolomics and the microbiome - becoming one of the few studies placing pathway research into a broader picture of BP biology. As research advances, intersectional stud-

ies such as this will need to become commonplace and fitting into the overall paradigm of how genetic studies are validated by research interlaying findings from different studies, and extending their insights into translational research for hypertension (Figure 5).

From a clinical perspective, increased access to genetic data is providing many applications with clinical utility. One example is the use of GRSs for coronary heart disease (CHD) for prediction and prevention of disease [99]. Kullo et al conducted a clinical trial ($n = 203$) in participants with no CHD. Participants were randomized to receive a 10-year risk prediction using a clinical risk score (CRS) alone or a CRS and CHD GRS and results showed the latter group had lower cholesterol levels and were more likely to receive statins. This study had used shared decision making based on the patients overall risk [99]. Recently a study by Weale et al developed a PRS for atherosclerotic CVD including CHD [100]. They presented an integrated risk tool that performs 10-year risk prediction across diverse ethnic and ancestry groups. They found improved prediction of CHD across individuals of not only self-reported White ethnicities but Black/African, American/Black, Caribbean/Black, African and South Asian (Indian, Bangladeshi or Pakistani) ethnicities – with PRS effect sizes in these ethnicities being significant and of comparable size to those seen in individuals of White ethnicities [100]. These positive results provide for the first time a validated PRS tool that can generalize across ancestries and presents a roadmap example for similar studies that may be appropriate for informing hypertension treatment using BP GRSs.

The pharmacogenetics of BP drugs, understanding if a person's drug response depends on genetic influences, has also gained attention - but this is an area which has room for improvement before offering clinical potential. For example, Arnett et al developed the GenHAT (Genetics of Hypertension Associated Treatments) study, an investigation of hypertension genetic variants and their interactions with antihypertensive treatments in relation to CHD [101]. This study was conducted over 4.9 years in 39,114 hypertensive cases across ethnicities – with individuals grouped depending on their antihypertensive medication class – providing one of the largest and most diverse pharmacogenetic BP studies. However, the results were not as promising as expected, with, for example, results
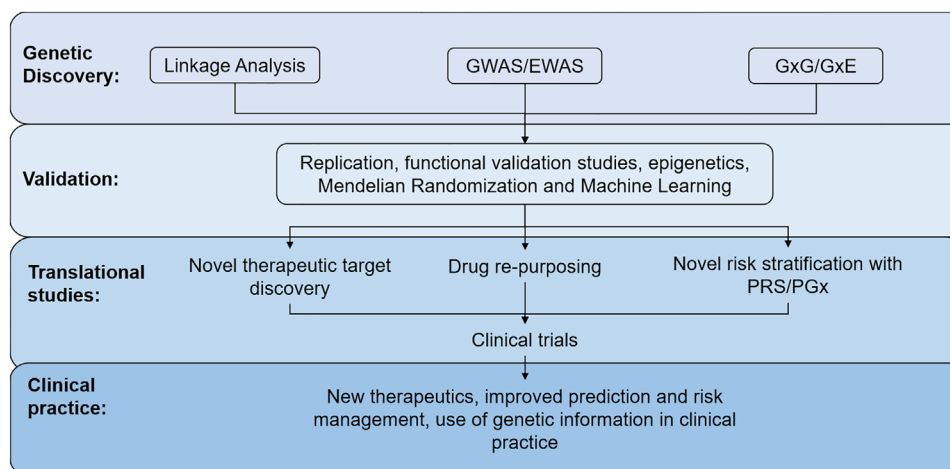
FIGURE 5 *Key concepts on the path from gene discovery to clinical practice. Discovery and validation of candidate genes was initially done using linkage analysis in families (success story for monogenic disorders); GWAS and EWAS are the current methods for gene discovery for essential hypertension. The discoveries from these experiments are being complemented and expanded upon with epigenetic functional investigations, with further discovery science and interpretation using GxG and GxE studies. For validation and interpretation of results to provide insights to causal mechanisms, approaches such as Mendelian randomization, and machine learning methods are being applied and functional studies of candidate genes in the laboratory. Validating hypertension mechanisms is leading to translational studies offering novel drug discovery, drug re-positioning, application of PRSs for better risk stratification and PGx studies*
Abbreviations: EWAS, epigenome-wide association study; GWAS, genome-wide association study; GxE, gene-environment interactions; GxG, gene-gene interactions; PGx, pharmacogenetics; PRS, polygenic risk scores.

exploring *AGT* gene interactions across hypertension drug classes finding 'no gene by treatment interactions to be statistically significant' [102]. This study and others suggest that the pharmaco-genetics of BP requires further work, which aligns with the overarching need for increased translational research before the genetics of BP can provide clinical benefit and advance hypertension treatments.

In conclusion, early genetic investigations of hypertension successfully established rodent models and advances in the human genome project has permitted discoveries for genes causing both monogenic forms of hypertension and EH. In recent years new lines of genetic analyses have taken shape, with epigenetics now being a hot topic. However, whilst the genetic insights and progress made so far have been vast, it is far from being comprehensive. There are now large whole genome datasets coming online and being collated into databases such as in gnomAD [103], the UK Biobank [55] and Genomics England's 100,000 genomes project [104], which provide full coverage of the genome at scale for the first time. Research

in hypertension is beginning to focus on non-European data, as translational findings need to benefit all populations equally and improve rather than worsen existing health disparities. Research also needs to expand in broader directions, spanning the metabolome, microbiome and incorporation of environmental factors. Such work will provide a more complete view of complex BP biology. As intersectional experiments develop, they may present an opportunity to connect findings with growing gene-gene and gene-environment research that may be the key to unlocking BP insights awaiting discovery. Overall, the genetics of hypertension is providing new information on underlying physiology, it is building towards an endgame for precision medicine, that may ultimately lead to lower BP as an important cofactor for decreasing CVD and potentially offer large global impact.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

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