Genomics of Hypertension

Sandosh Padmanabhan, Alisha Aman, Anna F Dominiczak

Affiliation:
BHF Glasgow Cardiovascular Research Centre
Institute of Cardiovascular and Medical Sciences
126 University Place
University of Glasgow

Corresponding Author:
Professor Dame Anna F Dominiczak DBE MD FRCP FAHA FRSE FMedSci
Regius Professor of Medicine
Vice-Principal and Head of College of Medical, Veterinary and Life Sciences
University of Glasgow
Wolfson Medical School Building
University Avenue
Glasgow G12 8QQ

Tel No: 0141 330 2738
E-Mail: Anna.Dominiczak@glasgow.ac.uk

Abstract

A complex network of interacting pathways involving renal, neural, endocrine, vascular and other mechanisms controls the main determinants of blood pressure - cardiac output and total peripheral resistance. Multiple genes within each of these systems contribute to the specialized functions regulating blood pressure. The monogenic forms of blood pressure dysregulation have provided valuable insights into blood pressure regulation and expanded our understanding of both the mechanisms and the treatment of hypertension. Genome wide association studies have identified over 100 single nucleotide polymorphisms associated with blood pressure phenotypes and have identified plausible novel pathways of BP regulation and putative drug targets.

From an epidemiological and clinical perspective, blood pressure (BP) at the higher end of the normal population distribution is associated with an increased risk of cardiovascular mortality and morbidity. Traditionally cardiovascular risk assessment is based on a predefined threshold at which the quantitative BP phenotype is converted into a binary trait (hypertension). Modern management strategies are directed towards BP reduction below this threshold at which the risk of excess cardiovascular events is reduced. Thus, the BP or hypertension (HTN) phenotype is a measurable characteristic of clinical risk, and discovering their genetic determinants will allow early risk prediction and targeted treatment. A complex network of interacting pathways involving renal, neural, endocrine, vascular, and other mechanisms controls the main determinants of BP - cardiac output and total peripheral resistance. Multiple genes within each of these systems contribute to the specialized functions regulating BP, and hence it is likely that many genes will participate in the development of HTN. Thus, by definition, BP is a complex trait that refers to any phenotype that does not exhibit classic Mendelian inheritance attributed to a single gene and results from the interactions between multiple genes and environmental factors. The distribution of BP in the population is a normal unimodal distribution supporting a complex multifactorial basis of BP regulation (1, 2). However, there are monogenic forms of HTN or hypotension ("Simple trait") which are very rare in the population and have little to no impact on public health, in contrast to essential HTN with a prevalence of ~27% amongst the adults worldwide. The monogenic forms of BP dysregulation have provided valuable insights into BP regulation and expanded our understanding of both the mechanisms and the treatment of HTN. The monogenic forms have also been the most successful examples of gene mapping with mutations in over 25 genes now linked to perturbed gene function and the BP dysregulation. The goal is to extend these successful examples to essential HTN.

Evidence for a genetic basis of essential hypertension

Whilst monogenic syndromes of HTN provide evidence that disruption of gene function can have a major impact on BP, to venture into formal genetic dissection of

BP or essential HTN requires evidence for a genetic contribution to these traits. Strong indication that BP and essential HTN may have a genetic component came from family studies demonstrating correlation of BP among siblings and between parents and children with part of this correlation attributable to genetic factors. Sir George Pickering noted a correlation coefficient of about 0.2 among BP of hypertensive propositi(3) and the relationship was similar to those of height. The Montreal adoption study(4) demonstrated correlation coefficients of 0.38 and 0.16 between biological and adoptive sibs, respectively, while the Victorian Family Heart Study(5) estimated correlation coefficients of 0.44 for non-twin siblings, 0.78 for monozygous twins, 0.50 for dizygous twins and 0.12 for spouse-spouse pairs. All these data indicate presence of a genetic component if the environmental influence is assumed to be similar between comparison groups. Hunt et al.(6) studied life table data for 94,292 persons and found the relative risks of developing HTN were 4.1 in men and 5 in women aged 20-39 who had at least two first degree relatives affected by HTN. Two additional measures that are commonly used to assess the genetic component of a trait are heritability (h^2), which is the fraction of variation in disease susceptibility due to genetic factors, and sibling recurrent risk (λs) which is the degree of elevated risk of disease for a sibling of an affected individual compared with a member of the general population. The heritability of clinic systolic BP is around 15-40% and 15-30% for clinic diastolic BP,(7, 8) whereas for ambulatory night-time systolic and diastolic BP the heritabilities are 32-70% and 32-50%.(7-11). It is pertinent to point out that though the heritability estimates are considerable, this does not equate to magnitude of genetic effect. This is because the denominator in the estimate of heritability comprises measurement error and variances attributable to genes, shared environment, non-shared environment and unmeasured determinants. Heritability is also a property of the population studied and low heritability estimates would suggest that genetic mapping would be difficult for that phenotype. The sibling recurrent risk of HTN is around 1.2-1.5(12) and taking this along with heritability and correlation estimates, the HTN and BP can be considered a trait with relatively modest genetic effect. As noted above, minimizing measurement error by using ambulatory recordings provide higher heritability estimates and using this phenotype can maximize the genetic signal (9-11).

Genetic architecture of blood pressure and hypertension

To successfully map the genetic basis of a trait, a good understanding of the genetic architecture of the trait is required. The genetic architecture of a trait refers to the number of disease variants that exist, their allele frequencies, the risks that they confer, and the interactions between multiple genetic and environmental factors. Mutations that account for Mendelian forms of HTN are highly penetrant and are usually under very strong selection, which keeps them at low frequencies with high levels of allelic heterogeneity. In contrast, susceptibility variants involved in essential HTN are likely to have low or medium penetrance and are probably not subject to such strong selection resulting in lower allelic heterogeneity. There is an ongoing debate whether common or rare variants contribute to essential HTN. Single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF)>1% account for more than 90% of the genetic differences between individuals and thus are likely to contribute to the population variation in BP rather than rare variants. This is the basis of the common disease/common variant (CDCV) hypothesis which states that genetic variants underlying complex traits occur with a relatively high frequency, have undergone little or no selection in earlier populations and are likely to date back to more than 100 000 years ago (13). Indeed from an evolutionary perspective, essential HTN is a disease of civilization and may be an undesirable pleiotropic effect of a preserved genotype that could have optimized fitness in the ancient environment (14). It is well recognized that HTN occurs earlier and with more severity in people of African ancestry compared to those of European ancestry (15). Differing predispositions to HTN in different populations may simply reflect different evolutionary selection pressures (“bottlenecks”) and the fact that populations do not share the same ancestral histories. In addition, an allele with no effect on reproductive fitness is expected to achieve high equilibrium frequency and this is likely to be the case for genes influencing HTN. The other competing model for HTN is the common disease rare variant hypothesis, with an inverse relationship between the magnitude of genetic effect and allele frequency. This model argues that diseases are common because of highly prevalent environmental influences, not because of common disease alleles in the population. Recently support for this has come from studies of rare variants of three genes namely Solute Carrier Family 12

Members 1 and 3 (*SLC12A1*, *SLC12A3),* and Potassium Voltage-Gated Channel Subfamily J Member 1 (*KCNJ1)* (major mutations of which cause Gitelman syndrome, and Bartter syndrome type 1 and 2 respectively) in the general population producing clinically significant BP reduction and protection from development of HTN (16). The most likely scenario would be that the allelic spectrum of the disease variants is the same as the general spectrum of all disease variants. Under this neutral model, although most susceptibility variants are rare with minor allele frequencies (MAF) <1%, SNPs with MAF>1% would account for more than 90% of the genetic differences between individuals. It is plausible that these common variants might contribute significantly to those common diseases in which susceptibility alleles might not be under intense negative selection. For the genome as a whole, it has been predicted that of the expected 10-15 million SNPs with MAF>1%, approximately half have MAF>10%. Given that the number of disease variants conferring mild to moderate risks might be large, there are likely to be hundreds of common and rare variants contributing to the familial clustering of HTN.

Monogenic forms of hypertension and hypotension

Table 1 summarises the rare monogenic forms of HTN that have contributed to our understanding BP regulation and targeted treatment (17, 18). We briefly describe each of the syndromes below.

Glucocorticoid-Remediable Aldosteronism or Familial Hyperaldosteronism Type 1

This rare autosomal dominant disorder is caused by the formation of a chimeric gene due to unequal crossing during meiosis. This results in aldosterone secretion stimulated by ACTH instead of angiotensin II resulting in HTN in childhood and early adulthood due to the secretion of ACTH as a fusion product of 5’ end of Cytochrome P450 Family 11 Subfamily B Member 1 (*CYP11B1)* and the distal coding sequence of member 2 of the same family (*CYP11B2) (19)*. Affected individuals are treated with a low-dose of glucocorticoids to supress ACTH secretion, amiloride to block ENaC, or spironolactone to prevent aldosterone binding to the mineralocorticoid receptor.

Apparent Mineralocorticoid Excess

Normally cortisol has mineralocorticoid activity which does not manifest as cortisol is metabolised promptly into cortisone by 11-Beta-Hydroxysteroid Dehydrogenase Type 2 (*HSD11B2*). In Apparent Mineralocorticoid excess (AME), absence or reduced activity of HSD11B2 results in cortisol acting as a potent mineralocorticoid. HSD11B2 activity is reduced by loss of function mutations and from excess ingestion of licorice whose active constituent is the glycyrrhizic acid. Treatment is with spironolactone which blocks the binding of cortisol to mineralocorticoid receptor and a low sodium diet (20).

Pseudohypoaldosteronism type II (Gordon's syndrome)

This syndrome is caused by mutations in the WNK Lysine Deficient Protein Kinase 1 and 4 (*WNK1*, *WNK4*), Kelch Like Family Member 3 (*KLHL3),* and Cullin 3 (*CUL3)*. Mutations in the WNK genes cause a disruption in the signalling pathway that regulates the BP by controlling the ion cotransporters NCC and NKCC2 in the kidney. *CUL3* and *KLH3* mutations, on the other hand, inhibit the ubiquitylation of WNK4 and other WNK isoforms leading to an over-activation of NCC/NKCC2 and thereby increased salt retention and HTN (21) (22). Affected individuals or subjects are treated with either a low-salt diet or thiazide diuretics which block NCC.

Liddle syndrome

This is an autosomal dominant condition with low levels of aldosterone and renin. Normally, a regulatory repressor called Nedd4-2 : Neural Precursor Cell Expressed, Developmentally Down-Regulated 4-Like, E3 Ubiquitin Protein Ligase (*NEDD4L*) binds to the sodium channel ENaC and promotes its degradation (23). Mutations in Sodium Channel Epithelial 1 Beta and Gamma Subunits *(SCNN1*and *SCNN1G)*, coding for the β and γ subunits of ENaC respectively, disrupts this binding leading to the constitutive expression and prolongation of the half-life of ENaC resulting in increased sodium reabsorption, volume expansion, and HTN. Treatment is with amiloride or triamterene.

Bartter's syndrome

This syndrome leads to loss of salt with hypokalemic metabolic alkalosis, normal to low BP and increased renin and aldosterone levels. It is caused by the direct loss of function of NKCC2 or a secondary cause disrupting the functions of NKCC2-ROMK channel, Chloride Voltage-Gated Channel Kb (*CLCNKB*), Bartin and Calcium

Sensing Receptor (*CaSR*) in the TAL cells (24). Individuals with this syndrome exhibit an increased level of urine prostaglandin E2 and reduced susceptibility to angiotensin II and norepinephrine. Treatment is with a potassium supplementation, and use of cyclooxygenase inhibitors, ACE- inhibitors and potassium sparing diuretics.

Gitelman's syndrome

Loss of function mutations in the *SLC12A3*, producing NCC in the DCT cells, lead to Gitelman's syndrome, which has a prevalence of 1:40000. Treatment options include oral potassium and magnesium supplementations with adequate salt and water consumption, and prescription of indomethacin, amiloride and eplerenone (25).

Primary Aldosteronism

This syndrome is characterised by the constitutive production of aldosterone leading to HTN with hypokalemia and suppressed circulating renin. Gain of function mutations in the gene Potassium Voltage-Gated Channel Subfamily J Member 5 *(KCNJ5)* resulting in membrane depolarisation and enhanced aldosterone production has been associated with over 40% of these aldosterone-producing adenomas (APAs). Other implicated genes include ATPase Na+/K+ Transporting Subunit Alpha 1 (*ATP1A1)*, encoding the α1 subunit of Na^+/K^+-ATPase, in 7% of APAs; ATPase Plasma Membrane Ca2+ Transporting 3 *(ATP2B3)*, encoding Ca^{2+}-ATPases (*SERCA*); and Calcium Voltage-Gated Channel Subunit Alpha1 D *(CACNA1D)*, encoding an L-type Ca^{2+} channel, CaV1.3 (26); and other gain of function mutations in genes regulating Na^+, Ca^{2+}, CACNA1D and ATP1A1.

Phaeochromocytomas and paragangliomas

These are rare neuroendocrine tumours in the adrenal glands, and sympathetic and parasympathetic paraganglia. Phaeochromocytomas, inherited as an autosomal dominant trait, are a result of mutations in the Ret Proto-Oncogene *(RET)*, responsible for the normal development of neurons. Other implicated genes include Von Hippel-Lindau Tumor Suppressor *(VHL)*, Kinesin Family Member 1B *(KIF1Bbeta)*, Egl-9 Family Hypoxia Inducible Factor 1 (*PHD2)*, Succinate Dehydrogenase Complex Assembly Factor 2 *(SDHAF2*), and the genes encoding the four subunits of succinate dehydrogenase: Succinate Dehydrogenase Complex Iron Sulfur Subunit A, B, C, and D *(SDHA, SDHB, SDHC* and *SDHD)*. Heterozygous germline mutations in *SDHC* and *SDHD* are associated with paraganglioma type 1, 3

and 4 (27). Germline mutation in the *SDHB* gene is the only reliable predictor of malignant Paragangliomas. Type 2 multiple endocrine neoplasia (MEN 2) is a rare familial cancer syndrome caused by mutations in the *RET* gene and is associated with phaeochromocytoma.

Polygenic hypertension

Major advances in identifying common variants associated with BP and HTN arose from genome wide association studies (GWAS). GWAS are large scale association mapping making no assumptions of the genomic location or function of the causal variant and provide a comprehensive approach to testing the hypothesis that common alleles contribute to heritable phenotype variation. GWAS rely on the linkage disequilibrium (LD) or correlation patterns of SNPs with functional variants and, therefore, identified SNPs are usually proxies of untyped functional variants. A typical GWAS experiment consists of genotyping 500,000 to 1 million SNPs across the genome, as depending on the population this number of SNPs is adequate to interrogate 80% of common SNPs with MAF greater than 5%. To adjust for multiple testing and to decrease type I error (false-positive rates), the statistical burden of proof relies on stringent P-values usually $P<5x10^{-8}$. Table 2 summarises the GWAS results for BP and HTN in different ancestries. One successful GWAS for HTN used an extreme case-control design representing the top 2% and bottom 9% of the BP distribution. Combined with follow-up validation analyses in 19,845 cases and 16,541 controls, it confirmed a locus near the Uromodulin (*UMOD*) gene (28). *UMOD* is exclusively expressed in the kidney, suggesting that the discovered variant may influence sodium homeostasis. Additionally, rare nonsynonymous variants in salt-handling genes have been shown to have comparatively large effects on BP in the general population(16). It is striking that the signals from all the GWAS for HTN and BP do not contain genes from highly plausible pathways, for example, the renin-angiotensin-aldosterone pathway or epithelial sodium channels. An important limitation of GWASs is that genome-wide significant SNPs often merely tag but do not provide direct information on the causal variants. To translate those signals into biological function, follow-up studies are necessary. Another issue arising from GWAS studies is the small fraction of population variance of BP (<5%) and BP

heritability (~2%) that are explained by the collective effect of all the GWAS loci identified so far.(29, 30). The missing heritability (30) conundrum is not unique to BP genetics, but is observed in most of the common phenotypes.

Despite the increasing pace of discovery of variants associated with BP and HTN, the limited predictive utility of these variants either singly or as part of a composite risk score is striking (29). The population distribution of the number of BP increasing alleles with nearly similar allele frequencies is normally distributed, as each SNP is inherited independently and hence the number of individuals in the population expected to carry all harmful risk alleles would be vanishingly small. One way of maximising information about the genetic signals is to create a composite genetic risk score coding for the presence or absence of risk alleles and their numbers for all the BP GWAS SNPs (29). In the ICBPGWAS (31), between the top and the bottom quintiles of the risk score a 4.6 mmHg systolic and 3.0 mmHg diastolic BP difference was detected, and the prevalence of HT was 29% compared to 16% in the top and bottom deciles. The score was also associated with early and advanced target organ damage including left ventricular hypertrophy, stroke and coronary artery disease but not chronic kidney disease or markers of renal function (32). The lack of association between BP risk score and kidney function may reflect just an association with GFR but not with other renal phenotypes such as albuminuria, or may indicate that high BP and renal disease do not necessarily have the same molecular origin. Using panels of genetic markers to predict risk has very poor discrimination and that the utility of GWAS approaches are primarily in identification of novel pathways. Below we describe some examples of plausible pathways identified by GWAS studies.

Functional characterisation of GWAS loci - Novel pathways

Uromodulin

Uromodulin is a protein expressed exclusively in the thick ascending limb of the loop of Henle (TAL) and encoded by the gene *UMOD*. Although the specific function is unknown, knockout mice demonstrate an increased localisation of NKCC2 in the subapical vesicles of TAL cells with reduced phosphorylation (33). This results in reduced co-transporter activity and greater sodium excretion. Alternatively,

overexpression of *Umod* causes a dose dependent increase in the excretion of the protein, associated with an elevation of BP. A GWAS of BP extremes linked an *UMOD* SNP rs13333226 to HTN, with the minor G allele conferring a lower risk of HTN and reduced urinary UMOD excretion (28). Furosemide, a loop diuretic and specific blocker of NKCC2, has been shown to enhance natriuresis and reduced BP in both transgenic mice and hypertensive individuals homozygous for the A allele of this SNP (34).

Natriuretic peptide

Polymorphisms in the ANP and BNP propeptides encoding genes, Natriuretic Peptide A and B (*NPPA*, *NPPB)*, have been associated with natriuretic peptides and BP (35, 36). Healthy individuals who are homozygous for the risk allele of SNP rs5068, present in the 3'-UTR of *NPPA*, were found to have lower expression of ANP propeptide, NT-proANP, possibly mediated by miR-425. This effect on circulating NT-pro-ANP is comparable to the environmental change induced by switching from an extremely low salt diet of 230mg/dl to 4600mg/dl (37). Natriuretic Peptide Receptor 3 *(NPR3)* is another plausible candidate identified by GWAS. This gene encodes for the natriuretic peptide receptor C/guanylate cyclase C (32, 38); KO studies have found the mutant mice to have lower BP due to the reduced clearance of the natriuretic peptides (39) . In a GWAS conducted for HTN, SNPs nearby this gene- rs1173771 has been associated with BP in Europeans (32) while rs1173766 in east Asians (38) .

SH2B Adaptor Protein 3 (SH2B3)

SH2B adaptor protein 3 is an intracellular adaptor protein expressed in the hematopoietic and endothelial cells. GWAS studies have found it to be a candidate gene for HTN and renal disease (40-43). Studies using mutant Dahl SS/MCW rats with a 6-bp deletion wherein the native proline-leucine-glutamate is replaced with a single glutamine, Sh2b3^{em1Mcwi}, resulted in the modification of the phosphotyrosine peptide binding pocket of the SH2 domain. This significantly reduced the BP of the mutant rat, along with reduced renal damage and blunted infiltration of the immune cells to the kidneys. Again, transplantation of the bone marrow from Sh2b3^{em1Mcwi} mutants to Dahl SS/MCW rats fed at a 2% NaCl salt rich diet led to a significant

decrease in the MAP and kidney injury (44). Further studies in KO Sh2b3 mice also have implicated the role of this gene in the development of HTN (45).

Cytochrome P450 Family 17 Subfamily A Member 1 (CYP17A1)

This gene encodes for cytochrome P450 which mediates the activity of 17a-hydroxylase in the biosynthesis of mineralocorticoids and glucocorticoids. It also mediates 17,20-lyase activity in the sex-steroid biosynthesis (35). Missense mutations in this gene is implicated in congenital adrenal hyperplasia with apparent mineralocorticoid excess, salt retention and HTN caused by the deficiency of 17a-hydroxylase. GWAS on HTN have found this gene to be associated with systolic BP (43).

Guanylate Cyclase 1 Soluble Subunit Alpha and Beta (GUCY1A3, GUCY1B3)

These genes encode the alpha and beta subunits of soluble guanylate cyclase (GC) which form a heterodimer to propagate NO signalling (46) (47). Activation of GCs also leads to the production of 3',5' cyclic GMP that blocks calcium influx and dephosphorylates myosin light chains, resulting in smooth muscle relaxation (48, 49). KO studies in mice have proved to cause HTN (50). GWAS on European ancestry have identified SNP rs13139571 on *GUCY1A3-1B3* to be associated with BP regulation (32).

Glutamyl Aminopeptidase (ENPEP)

This gene encodes for a glutamyl aminopeptidase converts angiotensin II to angiotensin III, which then activates the AT2 receptor promoting vasodilation. A nonsense variant, rs33966350, in a highly-conserved region of this gene, is predicted to result in a truncated protein with either a reduced enzymatic activity or a target to nonsense-mediated decay. This truncated protein is predicted to lead to a predominant angiotensin II signalling, which activates the AT1 receptor causing vasoconstriction, and ultimately result in elevated BP (51). This could be used as a plausible alternative therapeutic target to ACE inhibitors (32). *ENPEP* KO mice have been found to develop HTN (52). GWAS has identified SNP rs6825911 to be associated with BP in east Asians (38).

Other genes that are plausible candidates from GWAS include Pleckstrin Homology Domain Containing A7 (*PLEKHA7)* (43, 53-56); ATPase Plasma Membrane Ca2+

Transporting 1 *(ATP2B1)* (32) (57) which encodes for a plasma membrane calcium/calmodulin-dependent ATPase, which pumps calcium from the cytosol into the ECM; Solute Carrier Family 4 Member 7 *(SLC4A7)* which encodes for an electro-neutral sodium bicarbonate co-transporter (32); NADPH Oxidase 4 *(NOX4)* encodes for NADPH oxidase 4, which enhances vasodilation (58); Phosphodiesterase 5A (*PDE5A)* encodes for phosphodiesterase which is responsible for the hydrolysis of cGMP and is a target of hypertensive drug sildenafil (58). Solute Carrier Family 14 Member 2 (*SLC14A2),* a target of the calcium channel blocker drug nifedipine (58); Solute Carrier Family 8 Member A1 *(SLC8A1)* encodes for a Na^{+}-Ca^{2+} exchanger that alters cardiac contractility and hypertrophy and is expressed in the cardiomyocytes (58); Aldehyde Dehydrogenase 2 Family *(ALDH2,* mitochondrial*)* encodes for the mitochondrial enzyme alcohol dehydrogenase 2 for alcohol metabolism and has been associated with HTN through the modification of alcohol consumption (38).

Future Directions

Whilst high throughput genomic methods have resulted in the identification of robust signals for blood pressure, the translation into clinical applications have been limited. In this context, it is worthwhile recognising that GWAS studies are population-based studies focussing on associations at a population level. Other points to note are GWAS studies SNPs and phenotypes in isolation and has so far ignored pleiotropy, environmental interactions and gene-gene interactions. All these are crucial in the complex biology of blood pressure and the era of GWAS has laid the foundation of robust association study methods which now needs to progress towards studies of pathways and networks and biological function that would allow population level discoveries to be relevant on an individual level. Alongside this, efforts are ongoing on integrating other omics methods such as epigenomics and metabolomics.

Epigenomics and Hypertension

Epigenetics refers to heritable changes in gene expression that does not involve changes to the underlying DNA sequence. Epigenetic mechanisms involve modification of DNA through methylation, histone modification or effect of non-coding RNA. Unlike genomic variants, epigenetic alterations can be difficult to study as they are often tissue or cell-type specific. A genome wide methylation

study of leukocyte DNA in a small cohort of 8 African-American hypertensive subjects and 8 normotensive age-matched controls showed no significant associations, but there was one putative association between methylation of Sulfatase 1 (*SULF1*) gene and HTN(59). In another study of 60 hypertensive patients and controls, lower levels of 5mC were observed in DNA of patients with essential HTN (60). In animal models, a study of renal outer medullary tissue from the Dahl S hypertensive rat model and the SS.13BN26 congenic strain showed differential methylation in 80% of the CpG islands in response to salt (61).

Whilst these studies offer exploratory support for the role of epigenetics in HTN, the evidence that epigenetic changes can explain the missing heritability is lacking (62-67) and further studies are clearly needed to assess the causal implications of epigenetics in BP regulation.

Metabolomics

Metabolomics is the systematic study of metabolites, which are small molecules generated by the process of metabolism, and has been important in elucidating the pathways underlying metabolic disorders. Metabolomic profiling of over 3000 adult twins identified a putative novel pathway for BP regulation involving a dicarboxylic acid (hexadecanedioate) with a causal role supported by in vivo studies in rats (68). The role of hexadecanedioate in a vascular mechanism for HTN is supported by evidence from a study of pulmonary HTN, indicating a disruption of β-oxidation and an increase of ω-oxidation in this condition and pointing to a putative role in elevating pressure in both the systemic and the pulmonary circulations (69). The strongest genetic association seen with hexadecanedioate maps to Solute Carrier Organic Anion Transporter Family Member 1B1 (*SLCO1B1)*, an association previously reported in a metabolome-wide genetic study in Caucasians (70). Targeted metabolomics profiling in the European Prospective Investigation Into Cancer and Nutrition (EPIC)-Potsdam study showed higher concentrations of serine, glycine, and acyl-alkyl-phosphatidylcholines C42:4 and C44:3 tended to be associated with higher and diacyl-phosphatidylcholines C38:4 and C38:3 with lower predicted 10-year HTN-free survival (71). Other metabolite associations with incident HTN and BP come from two US studies which found 4-hydroxyhippurate, a metabolic sex steroids pattern and 2 diacylglycerols 16:0/22:5 and 16:0/22:6 to be associated with BP and incident HTN (72, 73). Finally, Menni *et al.* showed 12 metabolites to be strongly associated with

pulse wave velocity with uridine, phenylacetylglutamine, and serine appearing to strongly correlate with PWV in women (74).

Some important factors to be addressed in future studies of BP as a quantitative trait would be to more accurately model BP in subjects on antihypertensive treatment by considering the number of drugs, drug dosage and adherence as well as the use of longitudinal BP data. Novel strategies are needed to efficiently discover causal and clinically useful genetic markers. The next level of discovery will be more challenging as the molecular and functional dissection of the novel variants require more detailed functional studies in contrast to the high-throughput screening methods applied so far. The identification of a genomic approach to discover novel pathways and/or predict response (or lack of response) to existing anti-hypertensive drugs will be of benefit in developing the novel classes of drugs and may ultimately define an indication for them at an early stage in the treatment of HTN.

References

1. Guyton AC, Coleman TG, Fourcade JC, Navar LG. Physiologic control of arterial pressure. Bulletin of the New York Academy of Medicine. 1969;45(9):811-30.
2. Guyton AC, Coleman TG, Cowley AW, Liard J-F, Norman RA, Manning RD. Systems analysis of arterial pressure regulation and hypertension. Annals of Biomedical Engineering. 1972;1(2):254-81.
3. Pickering GW. THe genetic factor in essential hypertension*. Annals of Internal Medicine. 1955;43(3):457-64.
4. Mongeau J-G, Biron P, Sing CF. The Influence of Genetics and Household Environment upon the Variability of Normal Blood Pressure: The Montreal Adoption Survey. Clinical and Experimental Hypertension Part A: Theory and Practice. 1986;8(4-5):653-60.
5. Harrap SB, Stebbing M, Hopper JL, Hoang HN, Giles GG. Familial Patterns of Covariation for Cardiovascular Risk Factors in Adults: The Victorian Family Heart Study. American Journal of Epidemiology. 2000;152(8):704-15.
6. Hunt SC, Williams RR, Barlow GK. A comparison of positive family history definitions for defining risk of future disease. Journal of chronic diseases. 1986;39(10):809-21.
7. Kotchen TA, Kotchen JM, Grim CE, George V, Kaldunski ML, Cowley AW, et al. Genetic determinants of hypertension: identification of candidate phenotypes. Hypertension. 2000;36(1):7-13.
8. Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, et al. Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the framingham heart study. Hypertension. 2000;36(4):477-83.
9. Fagard R, Brguljan J, Staessen J, Thijs L, Derom C, Thomis M, et al. Heritability of conventional and ambulatory blood pressures. A study in twins. Hypertension. 1995;26(6 Pt 1):919-24.

10. Fava C, Burri P, Almgren P, Groop L, Hulthen UL, Melander O. Heritability of ambulatory and office blood pressure phenotypes in Swedish families. Journal of hypertension. 2004;22(9):1717-21.
11. Kupper N, Willemsen G, Riese H, Posthuma D, Boomsma DI, de Geus EJ. Heritability of daytime ambulatory blood pressure in an extended twin design. Hypertension. 2005;45(1):80-5.
12. Caulfield M, Munroe P, Pembroke J, Samani N, Dominiczak A, Brown M, et al. Genome-wide mapping of human loci for essential hypertension. Lancet. 2003;361(9375):2118-23.
13. Lander ES. The new genomics: global views of biology. Science. 1996;274(5287):536-9.
14. Neel JV. Diabetes Mellitus: A "Thrifty" Genotype Rendered Detrimental by "Progress"? American Journal of Human Genetics. 1962;14(4):353-62.
15. Burt VL, Whelton P, Roccella EJ, Brown C, Cutler JA, Higgins M, et al. Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988-1991. Hypertension. 1995;25(3):305-13.
16. Ji W, Foo JN, O'Roak BJ, Zhao H, Larson MG, Simon DB, et al. Rare independent mutations in renal salt handling genes contribute to blood pressure variation. Nat Genet. 2008;40(5):592-9.
17. Padmanabhan S, Caulfield M, Dominiczak AF. Genetic and molecular aspects of hypertension. Circ Res. 2015;116(6):937-59.
18. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. Cell. 2001;104(4):545-56.
19. Lifton RP, Dluhy RG, Powers M, Rich GM, Cook S, Ulick S, et al. A chimaeric 11 beta-hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. Nature. 1992;355(6357):262-5.
20. Cerame BI, New MI. Hormonal hypertension in children: 11beta-hydroxylase deficiency and apparent mineralocorticoid excess. J Pediatr Endocrinol Metab. 2000;13(9):1537-47.
21. Boyden LM, Choi M, Choate KA, Nelson-Williams CJ, Farhi A, Toka HR, et al. Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. Nature. 2012;482(7383):98-102.
22. Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, et al. Human hypertension caused by mutations in WNK kinases. Science. 2001;293(5532):1107-12.
23. Shimkets RA, Warnock DG, Bositis CM, Nelson-Williams C, Hansson JH, Schambelan M, et al. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. Cell. 1994;79(3):407-14.
24. Simon DB, Karet FE, Hamdan JM, DiPietro A, Sanjad SA, Lifton RP. Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. Nat Genet. 1996;13(2):183-8.
25. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, et al. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. Nat Genet. 1996;12(1):24-30.
26. Zennaro MC, Boulkroun S, Fernandes-Rosa F. An update on novel mechanisms of primary aldosteronism. J Endocrinol. 2015;224(2):R63-77.
27. Fishbein L. Pheochromocytoma and Paraganglioma: Genetics, Diagnosis, and Treatment. Hematol Oncol Clin North Am. 2016;30(1):135-50.
28. Padmanabhan S, Melander O, Johnson T, Di Blasio AM, Lee WK, Gentilini D, et al. Genome-wide association study of blood pressure extremes identifies variant near UMOD associated with hypertension. PLoS Genet. 2010;6(10):e1001177.
29. Padmanabhan S. Prospects for Genetic Risk Prediction in Hypertension. Hypertension. 2013;61(5):961.
30. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461(7265):747-53.
31. Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;478(7367):103-9.

32. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011;478(7367):103-9.
33. Mutig K, Kahl T, Saritas T, Godes M, Persson P, Bates J, et al. Activation of the bumetanide-sensitive Na+,K+,2Cl- cotransporter (NKCC2) is facilitated by Tamm-Horsfall protein in a chloride-sensitive manner. J Biol Chem. 2011;286(34):30200-10.
34. Trudu M, Janas S, Lanzani C, Debaix H, Schaeffer C, Ikehata M, et al. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. Nat Med. 2013;19(12):1655-60.
35. Newton-Cheh C, Johnson T, Gateva V, Tobin MD, Bochud M, Coin L, et al. Genome-wide association study identifies eight loci associated with blood pressure. Nat Genet. 2009;41(6):666-76.
36. Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, et al. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet. 2009;41(3):348-53.
37. Arora P, Wu C, Khan AM, Bloch DB, Davis-Dusenbery BN, Ghorbani A, et al. Atrial natriuretic peptide is negatively regulated by microRNA-425. J Clin Invest. 2013;123(8):3378-82.
38. Kato N, Takeuchi F, Tabara Y, Kelly TN, Go MJ, Sim X, et al. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. Nat Genet. 2011;43(6):531-8.
39. Matsukawa N, Grzesik WJ, Takahashi N, Pandey KN, Pang S, Yamauchi M, et al. The natriuretic peptide clearance receptor locally modulates the physiological effects of the natriuretic peptide system. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(13):7403-8.
40. Böger CA, Heid IM. Chronic Kidney Disease: Novel Insights from Genome-Wide Association Studies. Kidney and Blood Pressure Research. 2011;34(4):225-34.
41. Huan T, Esko T, Peters MJ, Pilling LC, Schramm K, Schurmann C, et al. A Meta-analysis of Gene Expression Signatures of Blood Pressure and Hypertension. PLoS Genetics. 2015;11(3):e1005035.
42. Huan T, Meng Q, Saleh MA, Norlander AE, Joehanes R, Zhu J, et al. Integrative network analysis reveals molecular mechanisms of blood pressure regulation. Molecular Systems Biology. 2015;11(4):799.
43. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet. 2009;41(6):677-87.
44. Rudemiller NP, Lund H, Priestley JRC, Endres BT, Prokop JW, Jacob HJ, et al. Mutation of SH2B3 (LNK), a GWAS candidate for hypertension, attenuates Dahl SS hypertension via inflammatory modulation. Hypertension. 2015;65(5):1111-7.
45. Dale BL, Madhur MS. Linking Inflammation and Hypertension via LNK/SH2B3. Current opinion in nephrology and hypertension. 2016;25(2):87-93.
46. Baker DA, Kelly JM. Structure, function and evolution of microbial adenylyl and guanylyl cyclases. Molecular Microbiology. 2004;52(5):1229-42.
47. Yamagami S, Suzuki N. Diverse Forms of Guanylyl Cyclases in Medaka Fish – Their Genomic Structure and Phylogenetic Relationships to those in Vertebrates and Invertebrates. Zoological Science. 2005;22(8):819-35.
48. Ignarro LJ, Harbison RG, Wood KS, Kadowitz PJ. Activation of purified soluble guanylate cyclase by endothelium-derived relaxing factor from intrapulmonary artery and vein: stimulation by acetylcholine, bradykinin and arachidonic acid. Journal of Pharmacology and Experimental Therapeutics. 1986;237(3):893.
49. Murad F. Cyclic guanosine monophosphate as a mediator of vasodilation. Journal of Clinical Investigation. 1986;78(1):1-5.
50. Friebe A, Mergia E, Dangel O, Lange A, Koesling D. Fatal gastrointestinal obstruction and hypertension in mice lacking nitric oxide-sensitive guanylyl cyclase. Proceedings of the National Academy of Sciences. 2007;104(18):7699-704.

51. te Riet L, van Esch JHM, Roks AJM, van den Meiracker AH, Danser AHJ. Hypertension. Circulation Research. 2015;116(6):960.
52. Ishii M, Hattori A, Numaguchi Y, Ma X, Nagasaka T, Tsujimoto M, et al. The effect of recombinant aminopeptidase A (APA) on hypertension in pregnant spontaneously hypertensive rats (SHRs). Early Human Development.85(9):589-94.
53. Fox ER, Young JH, Li Y, Dreisbach AW, Keating BJ, Musani SK, et al. Association of genetic variation with systolic and diastolic blood pressure among African Americans: the Candidate Gene Association Resource study. Human Molecular Genetics. 2011;20(11):2273-84.
54. Ho JE, Levy D, Rose L, Johnson AD, Ridker PM, Chasman DI. Discovery and replication of novel blood pressure genetic loci in the Women's Genome Health Study. J Hypertens. 2011;29(1):62-9.
55. Hong K-W, Jin H-S, Lim J-E, Kim S, Go MJ, Oh B. Recapitulation of two genomewide association studies on blood pressure and essential hypertension in the Korean population. J Hum Genet. 2010;55(6):336-41.
56. Lin Y, Lai X, Chen B, Xu Y, Huang B, Chen Z, et al. Genetic variations in CYP17A1, CACNB2 and PLEKHA7 are associated with blood pressure and/or hypertension in She ethnic minority of China. Atherosclerosis. 2011;219(2):709-14.
57. Monteith GR, Kable EPW, Kuo TH, Roufogalis BD. Elevated Plasma Membrane and Sarcoplasmic Reticulum Ca2+Pump mRNA Levels in Cultured Aortic Smooth Muscle Cells from Spontaneously Hypertensive Rats. Biochemical and Biophysical Research Communications. 1997;230(2):344-6.
58. Warren HR, Evangelou E, Cabrera CP, Gao H, Ren M, Mifsud B, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. Nat Genet. 2017;49(3):403-15.
59. Wang X, Falkner B, Zhu H, Shi H, Su S, Xu X, et al. A genome-wide methylation study on essential hypertension in young African American males. PLoS One. 2013;8(1):e53938.
60. Smolarek I, Wyszko E, Barciszewska AM, Nowak S, Gawronska I, Jablecka A, et al. Global DNA methylation changes in blood of patients with essential hypertension. Med Sci Monit. 2010;16(3):CR149-55.
61. Liu Y, Liu P, Yang C, Cowley AW, Jr., Liang M. Base-resolution maps of 5-methylcytosine and 5-hydroxymethylcytosine in Dahl S rats: effect of salt and genomic sequence. Hypertension. 2014;63(4):827-38.
62. Wang X, Prins BP, Sober S, Laan M, Snieder H. Beyond genome-wide association studies: new strategies for identifying genetic determinants of hypertension. Curr Hypertens Rep. 2011;13(6):442-51.
63. Wang X, Snieder H. Genome-wide association studies and beyond: what's next in blood pressure genetics? Hypertension. 2010;56(6):1035-7.
64. Friso S, Carvajal CA, Fardella CE, Olivieri O. Epigenetics and arterial hypertension: the challenge of emerging evidence. Transl Res. 2015;165(1):154-65.
65. Kotchen TA, Cowley AW, Jr., Liang M. Ushering Hypertension Into a New Era of Precision Medicine. JAMA. 2016;315(4):343-4.
66. Liang M, Cowley AW, Jr., Mattson DL, Kotchen TA, Liu Y. Epigenomics of hypertension. Semin Nephrol. 2013;33(4):392-9.
67. Cowley AW, Jr., Nadeau JH, Baccarelli A, Berecek K, Fornage M, Gibbons GH, et al. Report of the National Heart, Lung, and Blood Institute Working Group on epigenetics and hypertension. Hypertension. 2012;59(5):899-905.
68. Menni C, Graham D, Kastenmuller G, Alharbi NH, Alsanosi SM, McBride M, et al. Metabolomic identification of a novel pathway of blood pressure regulation involving hexadecanedioate. Hypertension. 2015;66(2):422-9.
69. Zhao Y, Peng J, Lu C, Hsin M, Mura M, Wu L, et al. Metabolomic heterogeneity of pulmonary arterial hypertension. PLoS One. 2014;9(2):e88727.

70. Suhre K, Shin SY, Petersen AK, Mohney RP, Meredith D, Wagele B, et al. Human metabolic individuality in biomedical and pharmaceutical research. Nature. 2011;477(7362):54-60.
71. Dietrich S, Floegel A, Weikert C, Pischon T, Boeing H, Drogan D. Identification of Serum Metabolites Associated With Incident Hypertension in the European Prospective Investigation into Cancer and Nutrition-Potsdam Study. Hypertension. 2016;68(2):471-7.
72. Kulkarni H, Meikle PJ, Mamtani M, Weir JM, Barlow CK, Jowett JB, et al. Plasma lipidomic profile signature of hypertension in Mexican American families: specific role of diacylglycerols. Hypertension. 2013;62(3):621-6.
73. Zheng Y, Yu B, Alexander D, Mosley TH, Heiss G, Nettleton JA, et al. Metabolomics and incident hypertension among blacks: the atherosclerosis risk in communities study. Hypertension. 2013;62(2):398-403.
74. Menni C, Mangino M, Cecelja M, Psatha M, Brosnan MJ, Trimmer J, et al. Metabolomic study of carotid-femoral pulse-wave velocity in women. J Hypertens. 2015;33(4):791-6; discussion 6.

Table 1: Monogenic forms of hypertension

Locus	Position (GRCh38/ hg38)	Gene/ Nearest genes	Inheritance	Genomic and Phenotypic annotation	Therapeutic notes
1p36.13	16043752 - 16057308	*CLCNKB*	Autosomal Recessive	Bartter syndrome, type 3, OMIM #607364. Low blood pressure. Impaired chloride reabsorption in the thick ascending loop of Henle leads to impaired sodium reabsorption. Hypokalemic metabolic alkalosis. Increased plasma renin and aldosterone.	Sodium and potassium supplements Aldosterone inhibitors and angiotensin-converting enzyme (ACE) inhibitors Indomethacin
1p36.13	17018730 - 17054170	*SDHB*		Paragangliomas 4, OMIM #115310. Multiple catecholamine-secreting head and neck paragangliomas and pheochromocytomas. Adult onset.	Alpha adrenergic blockers for pheochromocytoma
1q23.3	161314376 - 161364745	*SDHC*	Autosomal Dominant	Paragangliomas 3, OMIM #605373. Tumours or extra-adrenal paraganglia associated pheochromocytoma.	Alpha adrenergic blockers for pheochromocytoma
2q36.2	224470150 - 224585397	*CUL3*	Autosomal Dominant	Pseudohypoaldosteronism, type IIE, OMIM *603136.	Thiazide diuretics

				Hypertension, Hyperkalemia, Hyperchloremic metabolic acidosis	
3p25.3	10141635 - 10153670	*VHL*	Autosomal Dominant	von Hippel-Lindau syndrome, OMIM #193300 Associated with retinal, cerebellar, and spinal hemangioblastoma, renal cell carcinoma (RCC), pheochromocytoma, and pancreatic tumours.	Alpha adrenergic blockers for pheochromocytoma
4q31.2	148078764 - 148442520	*NR3C2*	Autosomal Dominant Autosomal Dominant	Hypertension exacerbation in pregnancy, OMIM #605115. Missense mutation (S810L) in the mineralocorticoid receptor. Low-renin, low-aldosterone, hypokalemia. Progesterone and other steroids lacking 21-hydroxyl groups, normally MR antagonists, becoming potent agonists. Pseudohypoaldosteronism type I,OMIM #177735. Failure to thrive. Renal unresponsiveness to mineralocorticoids. Hyponatremia,	Spironolactone contraindicated sodium chloride treatment

				hyperkalemia, metabolic acidosis. Increased renin and aldosterone.	
5p15.3	218241 - 256699	*SDHA*		Paragangliomas 5, OMIM # 614165. Tumours or extra-adrenal paraganglia associated pheochromocytoma.	Alpha adrenergic blockers for pheochromocytoma
5q31.2	137617500 - 137736090	*KLHL3*	Autosomal Dominant/ Recessive	Pseudohypoaldosteronism, type IID, OMIM # 614495. Hyperkalemia. Hyperchloremic metabolic acidosis	Thiazide diuretics
7p22.3-7p22.1	10001 - 7239940		Autosomal Dominant	Familial Hyperaldosteronism type 2, OMIM #605635. Hyperaldosteronism due to adrenocortical hyperplasia not suppressed by dexamethasone.	
7q36.1	150991056 - 151014599	*ABP1, KCNH2, NOS3, ACCN3,*		NOS3 - Pregnancy induced Hypertension, OMIM +163729. Nitric Oxide plays an important role in the maintenance of cardiovascular and renal homeostasis	

8q24.3	142872357 - 142917843	*CYP11B1, CYP11B2*	Autosomal Dominant	Familial Hyperaldosteronism type 1 Glucocorticoid Remediable Aldosteronism (GRA), OMIM #103900. Chimeric gene. Plasma and urinary aldosterone responsive to ACTH; dexamethasone suppressible within 48 h.	Hypertension suppressed by dexamethasone
			Autosomal Recessive	Increased aldosterone and low renin. Corticosterone methyloxidase II deficiency, OMIM #61060. *CYP11B2*	Sodium chloride supplementation Fludrocortisone
			Autosomal Recessive	Enzymatic defect results in decreased aldosterone and salt-wasting, high plasma renin. Steroid 11β-hydroxylase deficiency, OMIM #202010. *CYP11B1* Neonatal onset. Virilisation, short stature, suppressed aldosterone and renin.	Glucocortioids to reduce the ACTH-driven adrenal hyperplasia and production of the various hormone precursors. Potassium-sparing diuretics.

10q11.2	43077069 - 43130349	*RET*	Autosomal Dominant	Multiple Endocrine Neoplasia, Type IIA, OMIM #171400. Associated with multiple endocrine neoplasms, including medullary thyroid carcinoma, pheochromocytoma, and parathyroid adenomas.	Alpha adrenergic blockers for pheochromocytoma
10q24.3	102830531 - 102837533	*CYP17A1*	Autosomal Recessive	17-Alpha-Hydroxylase Deficiency, OMIM #202110. Hypertension, hypokalemic alkalosis. Increased ACTH and FSH. Absent sexual maturation.	Glucocortioids to reduce the ACTH-driven adrenal hyperplasia and production of the various hormone precursors. Potassium-sparing diuretics.
11q12.2	61430125 - 61446767	*SDHAF2*	Autosomal Dominant	Paragangliomas 2, OMIM #601650. Tumours or extra-adrenal paraganglia associated pheochromocytoma.	Alpha adrenergic blockers for pheochromocytoma
11q23.1	112086847 - 112095794	*SDHD*	Autosomal Dominant	Paragangliomas 1, OMIM #16800. Tumours or extra-adrenal paraganglia associated pheochromocytoma.	Alpha adrenergic blockers for pheochromocytoma

11q24.3	128838020 - 128867373	*KCNJ1*	Autosomal Recessive	Bartter syndrome, antenatal, type 2, OMIM #241200. Reduced potassium recycling leads to impaired sodium reabsorption. Elevated plasma renin and aldosterone. Hypokalemia, hypochloremia, hyperprostaglandinuria	Sodium and potassium supplements Aldosterone inhibitors and angiotensin-converting enzyme (ACE) inhibitors Indomethacin
12p12.3 - 12p11.1	19847067 - 33147066		Autosomal Dominant	Hypertension with Brachydactyly Bilginturan syndrome, OMIM %112410. Brachydactyly, short phalanges, short metacarpals	
12p12.3	752923 - 911452	*WNK1*	Autosomal Dominant	Pseudohypoaldosteronism type IIC Gordon's syndrome, OMIM #614492. Gain-of-function mutations in *WNK1*. Hyperchloremic metabolic acidosis. Low plasma renin, normal or elevated K^+	Alkalizing agents, potassium-binding resins, prostaglandin inhibitors, and diuretics.
15q21.1	48206301 - 48304078	*SLC12A1*	Autosomal Recessive	Bartter syndrome, antenatal, type 1, OMIM #601678. Homozygous or compound heterozygous mutation in the sodium-potassium-chloride cotransporter-2 gene.	Sodium and potassium supplements Aldosterone inhibitors and angiotensin-

					converting enzyme (ACE) inhibitors Indomethacin
16p12.2	23302270 - 23216879	*SCNN1B, SCNN1G*	Autosomal Dominant	Liddle Syndrome, OMIM # 177200. Constitutive activation of epithelial sodium transporter, ENaC. Low plasma renin and aldosterone. Hypokalemia	Amiloride or Triamterene
16q13	56865207 - 56915850	*SLC12A3*	Autosomal Recessive	Gitelman syndrome, OMIM #263800. Low BP. Loss-of-function mutation leads to lower sodium reabsorption. Increased plasma renin. Renal potassium and magnesium wasting.	Potassium and magnesium supplements. NaCl intake.
16q22.1	67431133 - 67437551	*HSD11B2*	Autosomal Recessive	Apparent Mineralocorticoid Excess, OMIM # 218030. Increased plasma ACTH. Increased urinary cortisol/cortisone ratio. Low plasma renin and aldosterone.	Spironolactone
17q21.2	42780631 - 42797066	*WNK4*	Autosomal Dominant	Pseudohypoaldosteronism type IIB Gordon's syndrome, OMIM #614491. Loss-of-function mutations in *WNK4*. Low plasma renin, normal or elevated K^+	Alkalizing agents, potassium-binding resins, prostaglandin inhibitors, and diuretics.

Table 2: GWAS results for BP and Hypertension in different ancestries

Chr	SNP	Genotype	Coded allele	Coded allele frequency			BP effect			Nearest gene(s)
				European	Asian	African	European	Asian	African	
1p36.2	rs880315	C/T	C	0.35	0.59	0.16	↑	↑	-	*CASZ1*
1p36.22	rs17367504	A/G	G	0.17	0.10	0.06	↓	↓	-	*MTHFR, CLCN6, NPPA, NPPB*
	rs5068	C/T	C	0.07	0.00	0.01	↓	-	-	
1p13.2	rs2932538	C/T	C	0.73	0.80	0.85	↓	-	-	*SLC16A1, CAPZA1, ST7L, MOV10*
	rs17030613	A/C	C	0.19	0.45	0.05	-	↑	-	
	rs10745332	A/G	A	0.74	0.81	0.77	-	↑	-	
1q32.1	rs2169137	C/G	G	0.74	0.94	0.80	↑	-	-	*MDM4*
1q42.2	rs2004776	A/G	A	0.26	0.67	0.54	↑	-	-	*AGT*
2p23.2	rs1275988	A/G	A	0.60	0.23	0.08	↓	-	-	*KCNK3*
2q11.2	rs7599598	A/G	A	0.57	0.63	0.09	↓	-	-	*FER1L5*

2q24.3	rs1446468	A/G	A	0.53	0.47	0.96	↓	-	-	*FIGN*
	rs13002573	A/G	G	0.25	0.40	0.11	↓	-	-	*FIGN*
	rs16849225	C/T	C	0.75	0.59	0.94	-	↑	-	*FIGN*
	rs6749447	G/T	G	0.28	0.72	0.58	↑	-	-	*STK39*
2q32.1	rs16823124	A/G	A	0.23	0.51	0.10	↑	-	-	*PDE1A*
3p25.3	rs347591	G/T	G	0.33	0.23	0.51	↓	-	-	*HRH1-ATG7*
3p24.1	rs13082711	C/T	T	0.80	0.94	0.96	↓	-	-	*SLC4A*
	rs820430	C/T	T	0.64	0.40	1.00	↑	-	-	
3p22.1	rs9815354	A/G/T	A	0.23	0.12	0.17	↑	↑	-	*ULK4*
	rs3774372	C/T	T	0.77	0.87	0.81	↓	-	-	
	rs1717027	C/T	T	0.22	0.12	0.66	↑	-	-	
3p21.31	rs319690	A/G	A	0.51	0.75	0.41	↑	-	-	*MAP4*
	rs7651237	A/G	G	0.64	0.94	0.88	↑	-	-	

3p21.1	rs9810888	G/T	G	0.53	0.59	0.46	-	↑	-	*CACNA1D*
3q26.1	rs16833934	A/G	G	0.37	0.17	0.65	↓	-	-	*MIR1263*
3q26.2	rs419076	G/T	T	0.48	0.13	0.57	↑	-	-	*MECOM*
4q12	rs871606	A/G	A	0.87	0.78	0.76	↑	↑	-	*CHIC2*
4q21.21	rs16998073	A/T	T	0.19	0.30	0.05	↑	↑	-	*FGF5*
	rs1458038	A/G	A	0.27	0.34	0.05	↑	-	-	
4q24	rs13107325	A/C/T	T	0.10	0.00	0.00	↓	-	-	*SLC39A8*
4q25	rs6825911	C/T	C	0.20	0.48	0.54	-	↑	-	*ENPEP, PITX2*
4q32.1	rs13139571	A/C	C	0.74	0.68	0.88	↑	-	-	*GUCY1A3-GUCY1B3*
5p13.3	rs1173771	C/T	C	0.51	0.57	0.81	↑	-	-	*NPR3-C5orf23*
	rs7733331	C/T	T	0.50	0.42	0.42	↓	-	-	
	rs1173766	C/T	C	0.52	0.59	0.62		↑	-	
5q33.3	rs11953630	A/C/T	T	0.34	0.06	0.15	↓	-	-	*EBF1*

6p22.2	rs1799945	C/G	G	0.18	0.04	0.00	↑	↑	-	*HFE*
	rs198823	G/T	T	0.65	0.23	0.60	↓	-	-	
6p21.33	rs805303	C/T	C	0.70	0.57	0.30	↑	-	-	*BAG1*
	rs2021783	C/T	C	1.00	0.81	1.00	-	↑	-	*CYP21A2*
6p21.32	rs2854275	G/T	T	0.08	0.03	0.08	↓	-	-	*HLA-DQB1*
6p21.1	rs10948071	C/T	T	0.70	0.67	0.05	↓	-	-	*CRIP3*
6q22.33	rs13209747	C/G/T	T	0.45	0.48	0.12	↑	↑	↑	*RSPO3*
6q25.1	rs17080102	C/G	C	0.06	0.01	0.09	↓	↓	↓	*PLEKHG1*
7p15.2	rs17428471	G/T	T	0.08	0.05	0.14	↑	↑	↑	*EVX1-HOXA*
7p12.3	rs2949837	A/T	A	0.23	0.61	0.00	↑	-	-	*IGFBP3*
7q21.2	rs2282978	C/T	C	0.36	0.06	0.43	↑	-	-	*CDK6*
7q22.3	rs17477177	C/T	T	0.72	0.91	0.93	↓	-	-	*PIK3CG*
	rs12705390	A/G	G	0.72	0.91	0.93	↓	-	-	

7q36.1	rs3918226	C/T	T	0.10	0.00	0.00	↑	-	-	*NOS3*
8p23.1	rs4841569	A/G	G	0.57	1.00	0.89	↑	-	-	*BLK-GATA4*
	rs2898290	C/T	C	0.58	0.02	0.55	NR	-	-	
8q24.12	rs2071518	C/T	T	0.20	0.19	0.60	↑	-	-	*NOV*
10p12.31	rs11014166	A/T	A	0.63	0.97	0.89	↑	↓	-	*CACNB2*
	rs1813353	A/G	A	0.65	0.92	0.85	↑	-	-	
	rs4373814	C/G	G	0.63	0.53	0.43	↓	-	-	
	rs12258967	C/G	C	0.64	1.00	0.73	↑	-	-	
10q21.2	rs1530440	C/T	T	0.16	0.20	0.02	↓	-	-	*c10orf107*
	rs4590817	C/G	G	0.82	1.00	0.82	↑	-	-	
	rs12244842	G/T	T	0.25	0.19	0.35	↓	-	-	
	rs7070797	A/G	A	0.15	0.00	0.00	↓	-	-	
10q22.2	rs4746172	C/T	C	0.23	0.56	0.19	↑	-	-	*VCL*
10q23.33	rs932764	A/G	G	0.43	0.58	0.15	↑	-	-	*PLCE1*

10q24.32	rs1004467	C/T	T	0.92	0.67	0.81	↑	-	-	*CYP17A1-NT5C2*
	rs11191548	C/T	T	0.92	0.72	0.99	↑	↑	-	
	rs12413409	A/G	G	0.92	0.72	0.98	-	↑	-	
	rs4409766	C/T	T	0.93	0.78	0.82	-	↑	-	
	rs3824755	C/G	C	0.07	0.23	0.17	↓	-	-	
10q25.3	rs2782980	C/T	T	0.27	0.15	0.44	↓	-	-	*ADRB1*
	rs7076938	C/T	C	0.33	0.17	0.45	↓	-	-	
	rs1801253	C/G	G	0.32	0.15	0.41	↓	-	-	
11p15.5	rs661348	C/T	C	0.45	0.58	0.11	↑	-	-	*LSP1-TNNT3*
11p15.4	rs7129220	A/G	G	0.89	1.00	0.95	↓	-	-	*ADM*
11p15.1	rs381815	A/C/T	T	0.30	0.25	0.18	↑	-	-	*PLEKHA7*
	rs757081	C/G	G	0.63	0.66	1.00	↑	-	-	*PIK3C2A, NUCB2, NCR3LG1*

11p15.2	rs2014408	C/T	T	0.20	0.21	0.03	↑	↑	↑	*SOX6*
	rs4757391	C/T	C	0.18	0.17	0.23	-	↑	-	
11q13.1	rs4601790	A/G	G	0.25	0.42	0.07	↑	-	-	*EHBP1L1*
	rs3741378	A/G	A	0.15	0.38	0.36	↓	-	-	*RELA*
11q22.1	rs633185	C/G	G	0.68	0.55	0.82	↓	-	-	*FLJ32810-TMEM133*
11q24.3	rs11222084	A/T	T	0.40	0.07	0.26	↑	-	-	*ADAMTS8*
12q13.13	rs7297416	A/C	C	0.25	0.62	0.38	↓	-	-	*HOXC4*
12q21.33	rs11105354	A/G	G	0.12	0.42	0.11	↓	-	-	*ATP2B1*
	rs2681492	A/G	A	0.88	0.58	0.84	↑	-	-	
	rs2681472	C/T	T	0.88	0.58	0.89	↑	↑	-	
	rs17249754	A/G	G	0.88	0.59	0.84	↑	↑	-	
12q24.12	rs3184504	C/T	T	0.45	0.00	0.00	↑	-	-	*SH2B3*
	rs653178	A/G	A	0.56	1.00	1.00	↓	-	-	

12q24.13	rs11066280	A/T	T	1.00	0.75	1.00	-	↑	-	*RPL6-ALDH2*
12q24.21	rs35444	C/T	T	0.59	0.73	0.55	↑	-	-	*TBX5-TBX3*
	rs2384550	A/G	A	0.37	0.09	0.34	↓	-	-	
	rs10850411	C/T	T	0.72	0.46	0.66	↑	-	-	
	rs1991391	C/T	C	0.64	0.91	0.58	↑	-	-	
	rs11067763	A/G	A	0.89	0.61	0.65	-	↑	-	*MED13L*
15q21.1	rs1036477	A/G	G	0.10	0.42	0.61	↓	-	-	*FBN1*
15q24.1	rs6495122	A/C	A	0.38	0.78	0.78	↑	-	-	*CYP1A1-ULK3*
	rs1378942	G/T	G	0.32	0.79	1.00	↑	-	-	
15q24.2	rs11072518	C/T	T	0.34	0.42	0.44	↑	-	-	*COX5A*
	rs1133323	A/G	A	0.59	0.17	0.00	↓	-	-	
15q26.1	rs2521501	A/T	T	0.63	0.93	0.80	↑	-	-	*FURIN-FES*
16p12.3	rs13333226	A/G	G	0.18	0.05	0.38	↑	-	-	*UMOD*

16q22.1	rs33063	A/G	A	0.19	0.15	0.00	↑	-	-	*NFAT5*
17q21.31	rs12946454	C/T	T	0.71	0.62	0.80	↑	-	-	*PLCD3*
17q21.32	rs17608766	C/T	T	0.91	1.00	1.00	↓	-	-	*GOSR2*
17q21.33	rs12940887	C/T	T	0.41	0.09	0.03	↑	-	-	*ZNF652*
	rs16948048	A/G	G	0.42	0.09	0.42	↑	-	-	
20p12.2	rs1327235	A/G	G	0.52	0.48	0.53	↑	-	-	*JAG1*
	rs1887320	A/G	A	0.58	0.43	0.54	-	↑	-	
20q13.32	rs6015450	A/G	G	0.07	0.00	0.22	↑	-	-	*GNAS-EDN3*
	rs6092743	A/G	A	0.06	0.00	0.06	↑	-	-	*C20orf174*

Figure 1:

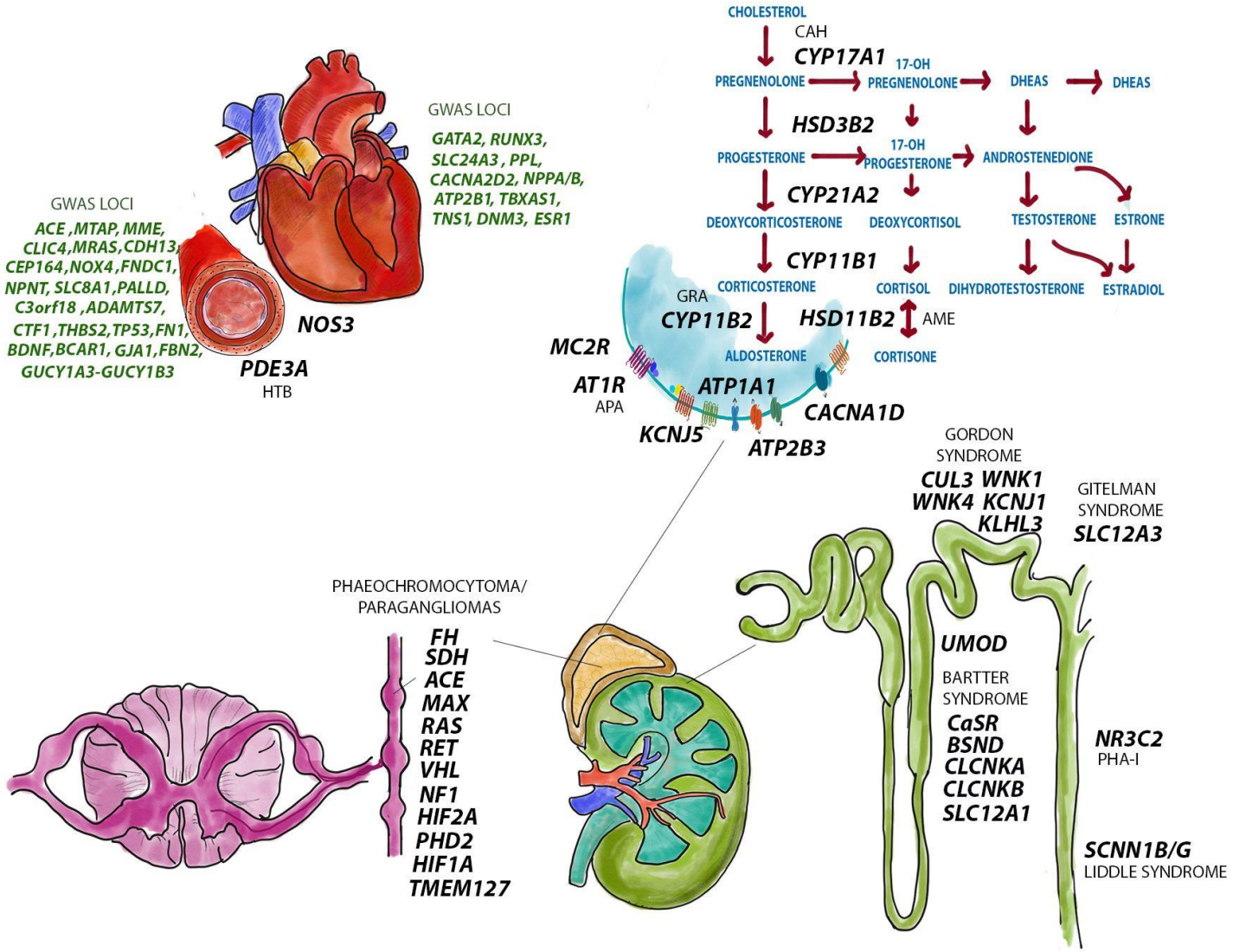
GWAS LOCI
GATA2, RUNX3,
SLC24A3 , PPL,
CACNA2D2, NPPA/B,
ATP2B1, TBXAS1,
TNS1, DNM3, ESR1
GWAS LOCI
ACE ,MTAP, MME,
CLIC4,MRAS,CDH13,
CEP164,NOX4,FNDC1,
NPNT, SLC8A1,PALLD,
C3orf18 ,ADAMTS7,
CTF1,THBS2,TP53,FN1,
BDNF,BCAR1, GJA1,FBN2,
GUCY1A3-GUCY1B3
NOS3
PDE3A
HTB
CHOLESTEROL
CAH
CYP17A1
PREGNENOLONE
17-OH PREGNENOLONE
DHEAS
DHEAS
HSD3B2
PROGESTERONE
17-OH PROGESTERONE
ANDROSTENEDIONE
CYP21A2
DEOXYCORTICOSTERONE
DEOXYCORTISOL
TESTOSTERONE
ESTRONE
CYP11B1
CORTICOSTERONE
CORTISOL
DIHYDROTESTOSTERONE
ESTRADIOL
GRA
CYP11B2
HSD11B2
AME
ALDOSTERONE
CORTISONE
MC2R
AT1R
APA
ATP1A1
CACNA1D
KCNJ5
ATP2B3
GORDON SYNDROME
CUL3 WNK1
WNK4 KCNJ1
KLHL3
GITELMAN SYNDROME
SLC12A3
UMOD
BARTTER SYNDROME
CaSR
BSND
CLCNKA
CLCNKB
SLC12A1
NR3C2
PHA-I
SCNN1B/G
LIDDLE SYNDROME
PHAEOCHROMOCYTOMA/
PARAGANGLIOMAS
FH
SDH
ACE
MAX
RAS
RET
VHL
NF1
HIF2A
PHD2
HIF1A
TMEM127

Figure 1: Genes and BP regulation. Genes linked to monogenic syndromes of high and low blood pressure are shown in bold black and genes that are putative loci from GWAS studies are in bold green. APA - aldosterone-producing adenoma; CAH – Congenital Adrenal Hyperplasia, HTB- Hypertension with Brachydactyly, GRA- Glucocorticoid Remediable Aldosteronism, PHA-I – PseudoHypoAldosteronism Type I,

Glossary of Terms

Phenotype

Phenotype or trait is the observable or measurable characteristic that is the target of genetic dissection.

Genetic Architecture

The genetic architecture of a trait refers to the number of distinct alleles that impact on the trait variation, their frequencies and penetrance.

Penetrance

Penetrance is the likelihood, or probability, that a particular genotype will be expressed in the phenotype. A penetrance of 100% means that the associated phenotype always occurs when the corresponding genotype is present, while a penetrance of 30% indicates that only 30% of those carrying a particular allele exhibit a phenotype.

Heritability

Heritability is the proportion of phenotypic variation due to the genetic differences between individuals within a population. Estimates of heritability can be used to describe the relative components of variance attributable to genetic factors and environmental factors. The heritability of a continuous trait is defined as the proportion of its total variance that is attributable to genetic factors in a particular population.

Linkage disequilibrium (LD)

LD can be simply defined as a non-random association between alleles at adjacent loci. That is, the presence of combination of alleles or markers in a population more often or less often than expected if the loci were segregating independently in the population. When a variant is first introduced into a population by mutation, it will be perfectly correlated with nearby variants, but over successive generations meiotic recombination will break up the correlations, and LD will decay. The LD between two markers within the same genomic region is commonly measured by the absolute value of D' and r^2. The higher the value of D', the lower the possibility that a

recombination event occurred between these two loci (*D'*=1 means that the two markers have not been separated by a recombination event). The absolute value of r^2 is more commonly used to quantify and compare LD in the context of mapping. When r^2=1, the two markers have not been separated by recombination and have the same allele frequency. In this case of perfect LD, the two markers are completely linked and observation at one marker provides complete prediction about the other.

Haplotype

Haplotype is a linear arrangement of closely linked alleles on the same chromosome that is inherited as a unit. Individual's genotypes at multiple tightly linked SNPs have two haplotypes, each containing alleles from one parent.

The International HapMap project

The international HapMap project (http://www.hapmap.org), has constructed genome-wide maps of LD patterns in multiple populations. One of the main objectives of the project is to identify set of SNPs that are in LD blocks to allow more efficient genotyping.

Hardy Weinberg Equilibrium (HWE)

The Hardy-Weinberg equilibrium states that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary influences.

Linkage studies

The concept of linkage analysis is to search for alleles or chromosomal segments that are shared by affected relatives more than expected by random Mendelian segregation. These segments are passed entirely from the parents to the offspring without recombination at meiosis. Linkage analysis is only carried out in families with affected relatives, and involves genotyping of several markers that spread over the entire genome. Markers that flank the disease gene or mutation tend to highly segregate with disease status in families. Identifying markers within such a segment that consistently accompanies the disease may indicate presence of susceptibility genetic factors nearby these markers. However, presences of such factors are neither necessary nor sufficient for the disease to develop.

Association Studies

Association mapping is based on the idea that genetic variants underlying complex traits occur with a relatively high frequency (>1%), have undergone little or no selection in earlier populations and are likely to date back to >100,000 years ago (common disease common variant hypothesis). Association analysis has potentially far greater power than linkage analysis for detecting variants with modest effect on disease risk, provided that the genetic marker is close enough to exhibit strong linkage disequilibrium (LD) with the functional variant. Unless targeting a specific, known polymorphism, all genetic association studies utilize one important population-genomic feature in their design: linkage disequilibrium (LD). LD is an extremely useful feature, as it means investigators do not need to genotype all polymorphisms in a region of interest. Instead, they can select a subset of SNPs that are proxies for the majority of all common genetic variation nearby (so-called "tagSNPs").

Genome-wide Association studies (GWAS)

Genome-wide association studies (GWAS) offer a hypothesis-free approach that systematically tests hundreds of thousands or more variants in the genome without prior knowledge of the location of the causal variants. GWA studies were made possible after assembling human genetic variants in large human genome reference projects such as the International HapMap Project, the Human Genome Project, and the 1000 Genome Project. For GWAS studies, large sample sizes are required to generate sufficient statistical power to overcome the multiple hypotheses that are tested. The high number of false-positive results are addressed by stringent multiple testing correction and seeking evidence from multiple replication and validation studies of the top signals. GWAS are also blind to rare and structural variants.