Kidney omics in hypertension: from statistical associations to biological mechanisms and clinical applications

OPEN

Maciej Tomaszewski[1,2], Andrew P. Morris[3], Joanna M.M. Howson[4], Nora Franceschini[5], James M. Eales[1], Xiaoguang Xu[1], Sergey Dikalov[6], Tomasz J. Guzik[7,8], Benjamin D. Humphreys[9], Stephen Harrap[10] and Fadi J. Charchar[10,11,12]

[1]Division of Cardiovascular Sciences, Faculty of Medicine, Biology and Health, University of Manchester, Manchester, UK; [2]Manchester Heart Centre and Manchester Academic Health Science Centre, Manchester University NHS Foundation Trust, Manchester, UK; [3]Centre for Genetics and Genomics Versus Arthritis, Centre for Musculoskeletal Research, Division of Musculoskeletal and Dermatological Sciences, University of Manchester, Manchester, UK; [4]Department of Genetics, Novo Nordisk Research Centre Oxford, Novo Nordisk Ltd, Oxford, UK; [5]Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, North Carolina, USA; [6]Division of Clinical Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee, USA; [7]Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; [8]Department of Internal and Agricultural Medicine, Jagiellonian University College of Medicine, Kraków, Poland; [9]Division of Nephrology, Department of Medicine, Washington University in St. Louis, St. Louis, Missouri, USA; [10]Department of Anatomy and Physiology, University of Melbourne, Melbourne, Victoria, Australia; [11]Health Innovation and Transformation Centre, School of Science, Psychology and Sport, Federation University Australia, Ballarat, Victoria, Australia; and [12]Department of Cardiovascular Sciences, University of Leicester, Leicester, UK

Hypertension is a major cardiovascular disease risk factor and contributor to premature death globally. Family-based investigations confirmed a significant heritable component of blood pressure (BP), whereas genome-wide association studies revealed >1000 common and rare genetic variants associated with BP and/or hypertension. The kidney is not only an organ of key relevance to BP regulation and the development of hypertension, but it also acts as the tissue mediator of genetic predisposition to hypertension. The identity of kidney genes, pathways, and related mechanisms underlying the genetic associations with BP has started to emerge through integration of genomics with kidney transcriptomics, epigenomics, and other omics as well as through applications of causal inference, such as Mendelian randomization. Single-cell methods further enabled mapping of BP-associated kidney genes to cell types, and in conjunction with other omics, started to illuminate the biological mechanisms underpinning associations of BP-associated genetic variants and kidney genes. Polygenic risk scores derived from genome-wide association studies and refined on kidney omics hold the promise of enhanced diagnostic prediction, whereas kidney omics-informed drug discovery is likely to contribute new therapeutic opportunities for hypertension and hypertension-mediated kidney damage.

Kidney International (2022) **102,** 492–505; https://doi.org/10.1016/j.kint.2022.04.045

Correspondence: *Maciej Tomaszewski, Division of Cardiovascular Sciences, Faculty of Medicine, Biology and Health, University of Manchester, Manchester, UK. E-mail: Maciej.Tomaszewski@manchester.ac.uk*

Received 12 December 2021; revised 10 March 2022; accepted 22 April 2022; published online 9 June 2022

KEYWORDS: blood pressure; gene expression; genetics; kidney

Increased blood pressure (BP) is a major cardiovascular risk factor: each 20– and 10–mm Hg increment in systolic BP (SBP) and diastolic BP (DBP), respectively, increases the risk of stroke and myocardial infarction by ≈2-fold.[1] Increased BP remains the single most important global

Editor's Note

Genetic mechanisms of blood pressure regulation are complex, but large-scale human genetic association studies combined with kidney multi-omic approaches are finally beginning to shine light on the inherited susceptibility to hypertension. In this review from the *Kidney International* Hypertension series, Tomaszewski *et al.* summarize the state of the field regarding integrative genomic approaches that can improve interpretation of genome-wide association studies for hypertension. Specific examples of successful mapping of new genetic susceptibility loci to causal variants, causal genes, and causal kidney cell types are also discussed.

contributor to premature death (ahead of smoking).[2] The kidney is an organ of "overriding dominance"[3,4] in BP regulation and the development of hypertension primarily through its intrinsic role in 2 main BP regulatory pathways (i.e., sodium and water homeostasis and the renin-angiotensin system [RAS]). Indeed, it is well established that the extracellular fluid volume expansion secondary to kidney defects in sodium excretion translates into BP elevation.[3] Renal juxtaglomerular cells synthesize renin (the rate-limiting enzyme of RAS) and release it into systemic circulation, whereas other kidney cells express the key elements of the RAS and contribute to BP regulation locally in an autocrine/paracrine manner.[5–7] Mutations in several genes expressed in the distal segments of the nephron, whose primary function is regulation of sodium homeostasis, have profound effects on BP.[8] Common genetic variants within some of these genes have also been associated with changes in BP, albeit their genetic effect is of lower magnitude.[9,10] Finally, key nonpharmacologic (e.g., dietary sodium restriction) and pharmacologic (e.g., diuretics and RAS inhibitors) interventions act, in part, through targeting kidney genes, molecules, and pathways. Common genetically mediated renal mechanisms of hypertension remain largely elusive, but their identification shows great promise to meet the pressing need[11] for novel therapies and prevention strategies.

Heritable nature of BP and hypertension

A family history of hypertension suggests a genetic component to BP regulation and takes 2 basic forms. Rarely, a low-frequency highly penetrant genetic mutation will produce a Mendelian pattern of inheritance. Mutations of this sort most commonly disturb the function of proteins with primary impact in the distal nephron to cause sodium retention and subsequent BP elevation of at least 10–20 mm Hg.[12] More commonly, a family history of hypertension simply reflects the broad quantitative correlation of BP between relatives.[13,14] Genetic influences herein are suggested by the stronger BP correlations seen between closer relatives.[14] Twin and family studies are frequently used to estimate narrow-sense heritability (h^2), defined as the additive "genetic" proportion of total BP variance.[15] For example, a large family study of relative pairs, including monozygotic and dizygotic twins, nontwin siblings, and parent-offspring pairs,[14] estimated the heritability of SBP to be 40% and that of DBP to be 46%. These values are consistent with analyses of family studies in other populations,[16] indicating that BP is a highly heritable trait.

The discovery of genes influencing BP depends on detecting significant differences in BP between contrasting genotypes. As a phenotype, BP is particularly challenging to measure accurately. First, it is an inherently variable, dynamic phenotype, unlike say height. This natural variability can be compounded if BP is measured in nonstandardized conditions (lying vs. sitting) and different methods (manual vs. semiautomatic). Care with measurement and standardization is essential for accurate phenotyping that, in turn, improves chances of gene discovery. To this end, higher number of measurements included in the approximation, higher reproducibility, no operator dependency,[17] elimination of "white-coat" cofounding, stronger correlation with the extent of hypertension-mediated organ damage, and higher narrow-sense heritability estimates make 24-hour ambulatory BP monitoring[18] a more attractive approach to phenotyping in genetic studies than clinic readings. However, resources with DNA profiles and information on BP from 24-hour ambulatory BP monitoring are scarce,[18] and because of that, almost all larger-scale population-based studies on genetics rely on clinic BP measurements.

Environmental exposures shared by relatives are an important component (13% of BP variance)[14] of family history. As such, identification and quantification of environmental factors allows partitioning of direct environmental effects on BP. Perhaps more important, it facilitates the discovery of environmental influences on gene expression through epigenetic mechanisms that might form an important element of heritability. Finally, the spirited debate between Platt and Pickering[19] regarding the nature and genetic explanation for the population distribution of BP was resolved in favor of Pickering's claim that the distribution was unimodal and that the quantitative BP variation was evidence of polygenic causation. In other words, an individual's BP is influenced by the inherited combination of genetic variants, each exerting a small effect on BP. Modern molecular analyses support this point and give estimates of the number and size of effect of the individual genetic variants.

Polygenic basis of BP and hypertension: genome-wide association studies

Genome-wide association studies (GWASs) typically interrogate large numbers (100,000s to millions) of genetic variants, predominantly single-nucleotide polymorphisms (SNPs), in thousands of participants, to find regions of the genome (loci) involved in common complex traits and diseases, such as BP regulation and hypertension (Figure 1a). There are now >1000 loci associated with BP or hypertension at stringent genome-wide significance ($P < 5 \times 10^{-8}$). These have been identified through efforts from large-scale consortia, such as the International Consortium of Blood Pressure Genetics and others, who have maximized statistical power to detect hypertension-associated genetic loci by amassing GWASs of BP traits (SBP, DBP, pulse pressure, mean arterial pressure, and hypertension) in the general population.[20–31] The largest published GWAS meta-analysis from International Consortium of Blood Pressure involved >1 million participants of European ancestry, including the UK Biobank and the US Million Veterans Program, and interrogated ≈7 million SNPs across the genome.[30]

GWASs have also been increasingly undertaken in non-European ancestry groups, albeit in smaller sample sizes because of the paucity of genetic data from populations of non-European ancestries. These investigations highlighted an overlap of BP loci across diverse populations and revealed

associations of variants more frequent in non-European ancestry populations.[25,28,32–34] For example, a BP GWAS in African and African American individuals (Continental Origins and Genetic Epidemiology Network - Blood Pressure [COGENT-BP] Study) identified 5 BP-associated SNPs, common only in African-ancestry populations, including rs76987554, an SNP at the TCF21 antisense RNA, inducing promoter demethylation gene (*TARID*).[28] Studies of Japanese individuals identified an SNP associated with pulse pressure at the cut-like homeobox 2 (*CUX2*) locus that has minor allele frequency <1% in populations of European ancestry.[34] Further research on variants that contribute to hypertension risk in populations of non-European ancestry is essential for genetic discovery and addressing health disparities.

Rare genetic variants (typically defined with minor allele frequency <1%) are generally not well captured by GWASs, and statistical power to detect associations with rare alleles is limited. However, opportunities for discovery of rare variant associations have been greatly enhanced through (i) targeted exome-array genotyping technology (designed to interrogate rare variants), (ii) improved reference panels that enable genome-wide imputation of variants to lower allele frequencies, and (iii) large cohorts (UK Biobank, Million Veteran Programme, and deCODE genetics). Around 90 rare variants have now been associated with BP or hypertension in multiple studies,[31,35–37] the largest of which included >1.3 million participants of diverse ancestry.[37] Most (>60%) of the rare variant associations mapped to loci that had been identified by common variant GWASs and, more important, helped to prioritize candidate genes at these loci. Several of these genes point to the importance of the kidney in BP regulation (such as angiotensinogen gene [*AGT*], glutamyl aminopeptidase [*ENPEP*], and phospholipase C epsilon 1 [*PLCE1*]).[37] Other new BP loci, uncovered through rare variant-based analyses, implicated genes strongly expressed in kidney (e.g., ATPase H^+ transporting V1 subunit G3 gene [*ATP6V1G3*]).[37] Only 4 genes (dopamine β-hydroxylase gene [*DBH*], nuclear receptor subfamily 3 group C member 2 [*NR3C2*], *AGT*, and phosphodiesterase 3A gene [*PDE3A*]) associated with BP regulation in the general population are known causes of monogenic conditions of high/low BP (i.e., dopamine β-hydroxylase deficiency, pseudohypoaldosteronism type 1A [renal], renal tubular dysgenesis, and brachydactyly with hypertension, respectively).[35–37] Most recently, whole exome sequencing (Figure 1a) in ≈450,000 participants found only 4 associations between rare variants and BP traits; one of them maps onto SLC9A3 regulator 2 gene (*SLC9A3R2*), which encodes a kidney-expressed scaffolding protein, with a potential role in sodium reabsorption.[38]

Collectively, genetic factors identified so far explain typically <6% of BP phenotypic variance.[30,37] Common BP-associated SNPs (minor allele frequency ≥5%) typically have modest effect (median, <0.25–mm Hg change in BP per risk allele copy), whereas the mean effects of rare variants are ≈7 times larger.[30,37] Most identified BP-associated variants map to non–protein-coding regions of the genome (e.g., intronic and intergenic regions), which presumably influence BP via regulation of gene expression as transcriptionally active SNPs (eSNPs). These variants may operate in a tissue-specific manner; their effect on expression of their targets (i.e., eGenes) may show a pattern specific to a given tissue or groups of tissues. Integration of BP GWASs with data from the Genotype Tissue Expression Project[39] has also revealed strong enrichment of BP eSNPs in tissues of the cardiovascular system, including arteries and the heart, as well as adrenal and adipose tissues.[30,37] However, the Genotype Tissue Expression Project has only a limited number of kidney samples; until recently, kidney has not been included in post-GWASs. This has hampered progress to understanding the genetic mechanisms of hypertension through regulation of gene expression in this essential organ.

What have we learned about BP and hypertension from kidney transcriptomics?

To compensate for the underrepresentation of kidney in Genotype Tissue Expression Project and other publicly available transcriptomics resources, recent collaborative efforts have established large collections of human renal tissues characterized by RNA sequencing for the purpose of multi-omics analyses.[7,37,40,41] The foundations of these collections are kidney tissue samples (collected after either elective cancer nephrectomies or kidney biopsy),[42–44] characterized at multiple molecular levels (e.g., DNA and RNA). This availability of matching sets of genotype and kidney gene expression profiles facilitates *cis*-expression quantitative trait loci (eQTL) analysis through which kidney eSNPs (SNPs associated with gene expression) and their partner kidney eGenes (genes related to eSNPs) are identified.[7,37,40,41] This kidney eQTL-derived information is then integrated with data from BP GWASs to further refine the signals of association with BP and identify most likely kidney mediator genes. Indeed, the convergence of genetic variants identified in BP GWASs with those showing effects on kidney expression mapped kidney genes onto approximately one-third of all known BP GWAS loci.[40] Approximately 50% of the genes revealed through the overlap of BP GWASs and kidney eQTL were shown to be due to the same shared underlying genetic variants using colocalization methods.[40] To demonstrate which of the colocalized kidney genes were causally associated to either increased or reduced BP, Mendelian randomization (MR) was applied.[40] In brief, MR uses genetic information (genotype) inherited at random (i.e., at conception) to determine whether a genetically predicted "exposure," such as a modifiable clinical risk factor (e.g., body weight) or molecular trait (e.g., gene expression or extent of DNA methylation at a given site), is causally linked to an "outcome" (e.g., SBP or DBP).[45] Many of the kidney genes with statistical evidence of potential causality to BP from MR have either undefined biological function or no prior biological connection to BP regulation or hypertension.[40] For example, solute carrier family 5 (sodium/inositol cotransporter), member 11 gene (SGLT6, *SLC5A11*)[40] is involved in D-glucose, D-xylose, and myoinositol transport

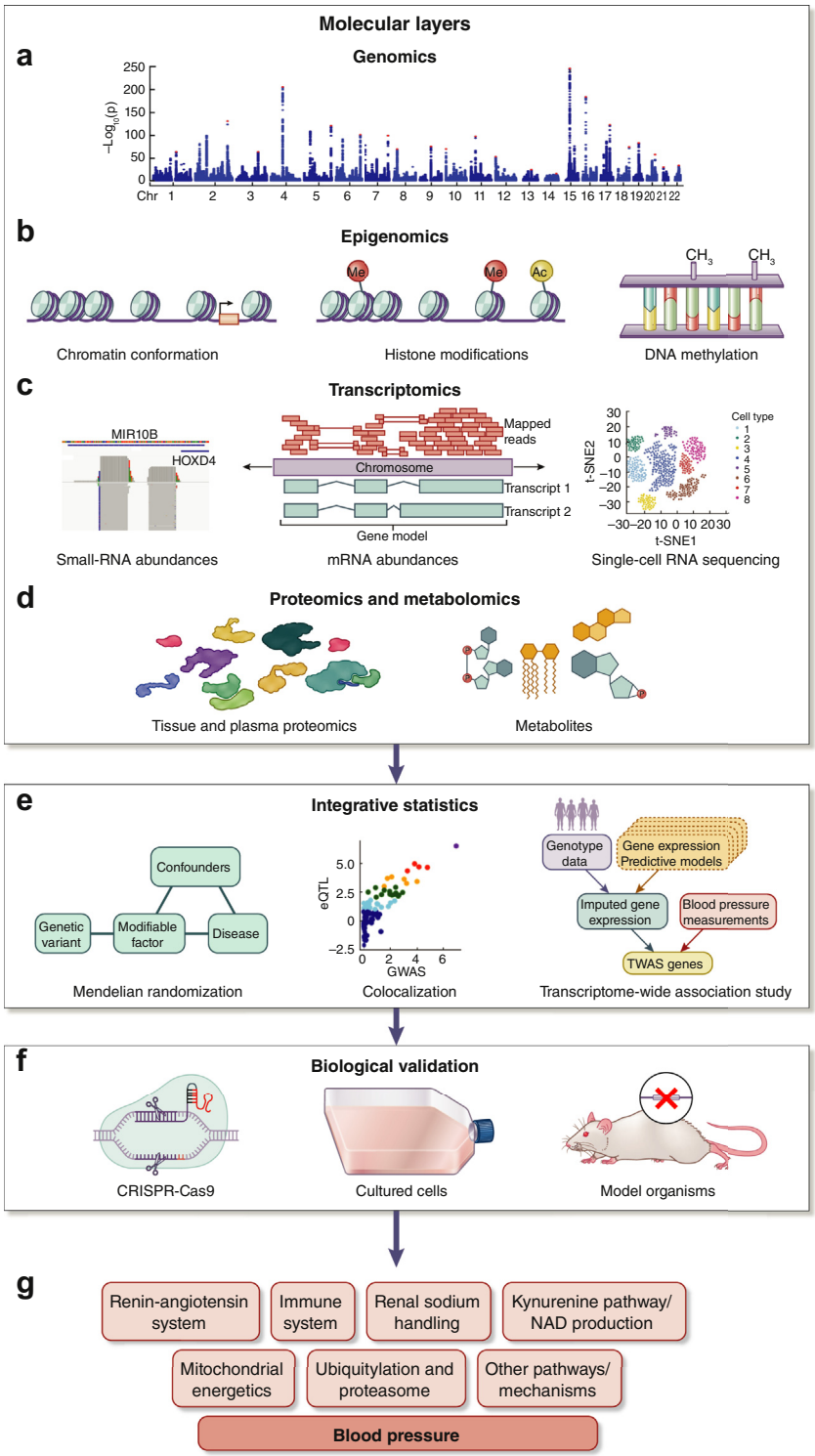

Figure 1 | Schematic representation of multi-omic approach to discovery of kidney genes involved in blood pressure (BP) regulation and hypertension. (**a**) Genomics data are generated by genome-wide genotyping arrays, exome arrays (targeting coding variants), and sequencing-based technologies that cover the whole genome (whole-genome sequencing) or just the coding regions (exome sequencing). A "Manhattan plot" visualizes the findings from analysis of associations between thousands of genetic variants and BP. (**b**) Epigenomic data are generated by array-based technologies or sequencing to quantify the extent of kidney DNA methylation (e.g., DNA-methylation array), histone modifications (e.g., chromatin immunoprecipitation sequencing), or open chromatin regions (e.g., assay for transposase-accessible chromatin sequencing). (**c**) Kidney transcriptome profiling is conducted through sequencing of, for example, poly-adenylated reverse-transcribed RNA (commonly referred to as RNA sequencing [RNA-seq]) to quantify, for example, abundance of renal mRNAs. Small RNA-seq involves physical isolation of small RNA fragments (<30 nucleotides) before sequencing. Microfluidics-based sequencing of reverse-transcribed RNA from single cells (single-cell RNA-seq) allows cell type–based analyses. (**d**) Proteomics data are generated by (continued)

in the kidney. SGLT6 was proposed to act through intrinsic resilience to osmotic stress via sodium-dependent cotransport of myoinositol[46]; its exact role in glucose metabolism is much less understood when compared with other members of this family (e.g., SGLT2). Adenosine diphosphate–ribosylation factor-like 3 gene (*ARL3*)[7,40] encodes a member of Arl family of guanosine triphosphate–binding proteins essential for signaling in the primary cilia.[47] Microtubule-associated protein 1B gene (*MAP1B*) encodes a component of cytoskeleton (Figure 2a). Interferon regulatory factor 5 gene (*IRF5*)[40] was implicated in the pathogenesis of autoimmune diseases, a response to viral infection and organ fibrosis,[48] whereas 3-hydroxyanthranilate 3,4-dioxygenase gene (*HAAO*)[40] is a part of the kynurenine pathway and the *de novo* synthesis of nicotinamide adenine dinucleotide (NAD).[49] Approximately 10% of kidney genes with the strongest evidence of potentially causal effect on BP are noncoding.[40] Most of these are long noncoding RNAs known to operate as chromatin modifiers, enhancers, or molecular sponges,[50] but the mechanisms underpinning their impact on BP remain to be discovered.

Apart from uncovering new genes, kidney transcriptomics has helped to strengthen or reprioritize the evidence supporting genes already known for their role in BP regulation or those previously associated with BP. For example, 2 key components of RAS (*AGT* and angiotensin-converting enzyme gene [*ACE*]) were implicated as the likely drivers of BP GWAS signals[9] on chromosomes 1 and 17, respectively, in the recent post-GWAS kidney omics analysis.[7] In contrast, angiotensin II converting enzyme 2 gene (*ACE2*; a key component of RAS responsible for converting angiotensin 1–9 to angiotensin 1–7) was not associated with hypertension in the recent differential gene expression analysis of >400 human kidneys.[44] A reduced kidney abundance of guanylate cyclase 1, soluble, α 3 gene (*GUCY1A3*) showed an association with BP increase[40] in MR studies, possibly via an effect on renal nitric oxide bioactivity and its downstream physiological consequences.[51,52] Other kidney genes known for their critical importance to regulation of sodium/water handling in the distal nephron (e.g., sodium channel epithelial 1 subunit β gene [*SCNN1B*] and sodium channel epithelial 1 subunit γ gene [*SCNN1G*] of epithelial sodium channel [ENaC]) emerged as potential contributors to hypertension-mediated effects of obesity on kidney disease in gene set enrichment analysis of the kidney transcriptome.[53] Some genes mapping to known BP GWAS loci were reprioritized on the basis of the insights from kidney transcriptomics (e.g., it was the expression of FES proto-oncogene, tyrosine kinase gene [*FES*] rather than furin, paired basic amino acid cleaving enzyme gene [*FURIN*] that showed a potentially causal effect on BP).[40] Indeed, one of the previous GWASs identified a signal of association between BP and genetic variation on chromosome 15 and reported it as the FURIN-FES locus.[22] Given its role as an endoprotease responsible for processing of precursor molecules of relevance to BP regulation (e.g., epithelial sodium channel[54] and B-type natriuretic peptide[55]) and the involvement in transforming growth factor-β1 cascade,[56] *FURIN* appeared as a biologically much stronger effector gene than its neighbor, *FES*.[22] The latter encodes a nonreceptor tyrosine kinase feline sarcoma oncogene, and its role in inflammatory response has only started to emerge.[57] Through an overlap between BP GWAS catalog and an in-house kidney *cis*-eQTL data set, *FES* was shown to partner (as kidney eGene) with BP GWAS variant on chromosome 15 (*rs2521501*); this was supported by both statistical colocalization analyses and MR.[40] Further support for *FES* as a potential mediator of association between locus on chromosome 15 and BP came from other kidney omics and subsequent MR studies.[40] In contrast, an initial weak signal of association between BP and *FURIN* in one of the kidney omics was not confirmed in follow-up MR studies.[40] Collectively, these data suggest that *FES* is a robust kidney eGene associated with BP. Of note, the same genetic locus on chromosome 15 implicated in GWAS of coronary artery disease colocalized also to *FES* (rather than *FURIN*) in eQTL-based analysis conducted in human coronary artery smooth muscle cells.[58]

Finally, integrating transcriptomic information from the kidney and >40 nonkidney human tissues available in Genotype Tissue Expression Project[39] demonstrated different patterns of kidney specificity of GWAS eSNP-eGene pairs.[40,59] This included GWAS eSNPs with transcriptional activity apparent only in the kidney (kidney eGene[s] only), GWAS eSNPs whose target eGenes have different identities between the kidney and nonkidney tissues, and eSNPs with kidney-specific allelic direction (the direction of the association with eGene expression is different between renal and nonrenal tissues).[40,59]

The analysis of the kidney "splice-ome" has also provided new insights into molecular mechanisms of hypertension by revealing that ≈10% of BP GWAS signals are associated with the expression of individual mRNA isoforms rather than total gene expression in the kidney (Figure 2c).[40] For example, genotypes of rs4582532 show association with renal expression of a splicing isoform of reduced NAD:ubiquinone oxidoreductase complex assembly factor 6 gene (*NDUFAF6*)

Figure 1 | (continued) multiple derivations of mass spectrometry, immunoassay (Olink), or aptamer-based approaches (SomaScan). Metabolite measurements can be generated by nuclear magnetic resonance spectroscopy. (**e**) Data from different biological layers are combined and examined using statistical colocalization, Mendelian randomization, and transcriptome-wide association studies. (**f**) Different laboratory-based strategies (e.g., CRISPR-Cas9) are used to confirm the effects of genetic variants/genetic regions/genes on the molecular and biological readouts *in vitro* and/or *in vivo*. (**g**) Statistically/experimentally validated kidney genes may map onto well-established pathways of BP regulation, such as renin-angiotensin system, and others may turn out to have a completely different and sometimes unexpected mode of action. Chr, chromosome; eQTL, expression quantitative trait locus; GWAS, genome-wide association study; NAD, nicotinamide adenine dinucleotide; t-SNE, t-distributed stochastic neighbor embedding; TWAS, transcriptome-wide association study.

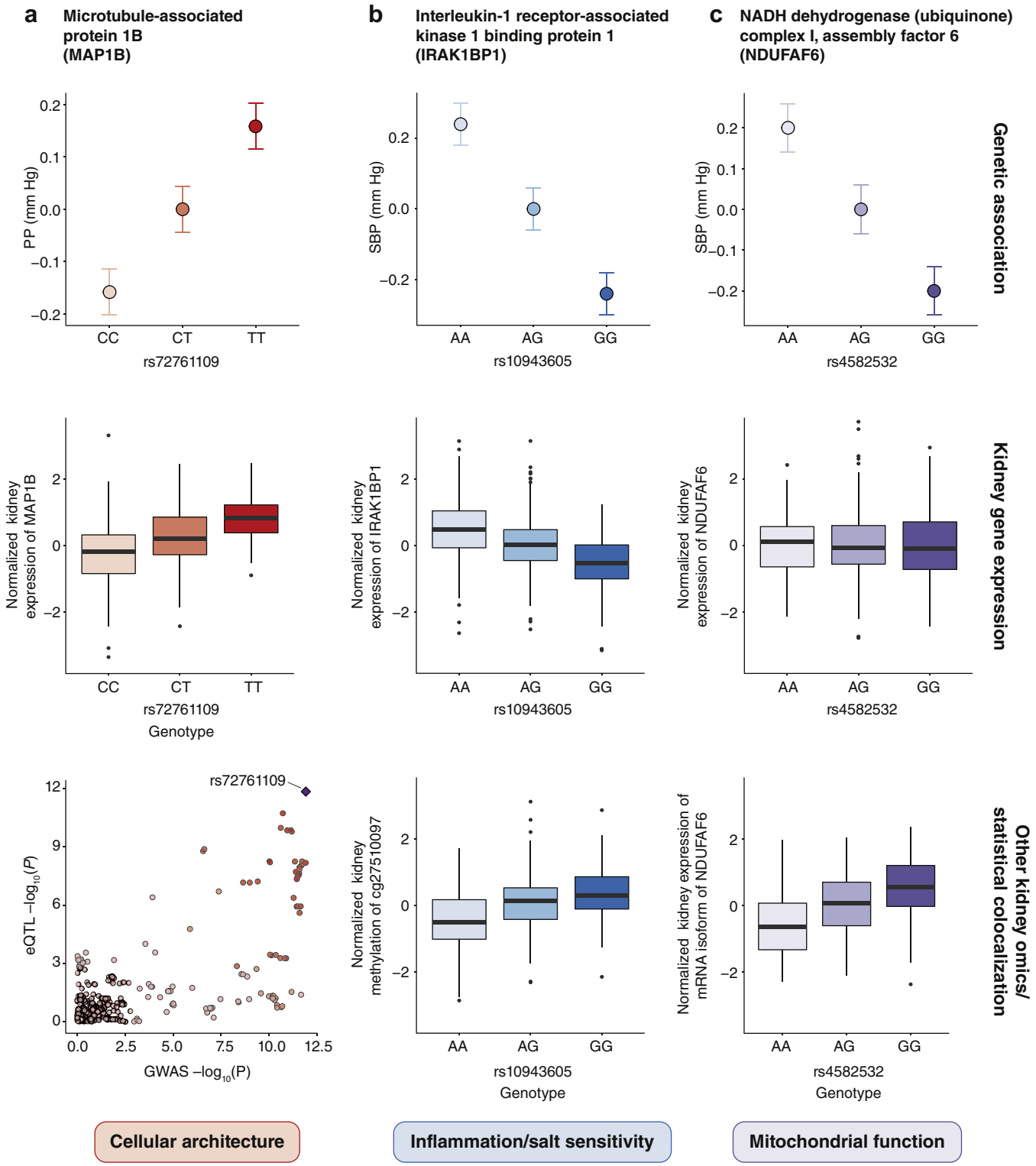

Figure 2| Examples of kidney genes showing causal association with blood pressure (BP): evidence from kidney omics. (**a**) A single-nucleotide polymorphism (SNP), rs72761109, was associated with pulse pressure (PP) in previous genome-wide association studies (GWASs). This sentinel BP GWAS SNP is transcriptionally active in the kidney; genotype of rs72761109 is associated with renal expression of microtubule-associated protein 1B gene (*MAP1B*) in *cis*-expression quantitative trait locus (*cis*-eQTL) analysis. Evidence for colocalization between kidney *cis*-eQTL signal and PP-GWAS signal. The *x* axis shows the negative $\log_{10}$ *P* value of the GWAS association, and the negative $\log_{10}$ *P* value for the *cis*-eQTL signal is shown on the *y* axis. The most significant colocalizing SNP is shown in purple and is labeled with its reference SNP ID. Other SNPs are colored by their linkage disequilibrium (r^2) with the most significant SNP (i.e., the darker color indicates the higher r^2). The colocalized gene (*MAP1B*) is known to play a role in cellular architecture. (**b**) The rs10943605 SNP was associated with systolic blood pressure (SBP) in previous GWASs. This variant is transcriptionally active in the kidney; genotype of rs10943605 is associated with renal expression (continued)

but not the total expression of this gene in the kidney. Another interesting example of such a BP GWAS variant is rs4750358, which maps in proximity of a weak acceptor splice site within a branch point sequence on chromosome 10.[40] Carriers of the BP-elevating genotype of this SNP have increased renal expression of a splicing mRNA isoform of BEN domain-containing 7 gene (*BEND7*) compared with noncarriers, whereas the total expression of the gene is unchanged across the genotypes.[40] Although the function of *BEND7* remains to be elucidated, it illustrates the emerging importance of splicing machinery operating in the kidney to genetic regulation of BP. It also suggests that eQTL-based analyses may not be sufficient to uncover the full spectrum of complex regulatory mechanisms underlying BP GWAS signals. Indeed, other types of RNAs (e.g., small noncoding RNAs) not captured routinely in standard RNA sequencing (Figure 1c) of the kidney are likely to mediate a certain proportion of associations identified by GWASs. Among them, microRNAs (miRs) are particularly important for the post-transcriptional control of gene expression and are increasingly recognized as key molecular players in hypertension and cardiovascular disease.[60–62] For example, miR-181a binds to the 3' untranslated region of renin mRNA in the kidney and downregulates renin while both circulating and renal miR-181a levels correlate with BP.[6] Given that a single miR can target many mRNAs and that both long noncoding RNAs (lncRNAs) and circular RNA are likely to mediate interactions between miRs and mRNAs,[63] profiling of all types of kidney RNAs will be necessary to fully understand their involvement in BP regulation and development of hypertension.

Finally, the integration of BP GWASs and human kidney transcriptomics revealed that, when compared with other complex human traits and diseases, BP phenotypes show strong enrichment for kidney eQTL signals.[40] Recent analyses showed that genes closest to rare BP variants were enriched among differentially expressed genes in the kidney.[37] The BP-associated variants are also overrepresented in kidney DNase I hypersensitive sites, which mark open chromatin.[37] Collectively, these data cemented the evidence for the key role of the kidney as a mediator of genetic predisposition to hypertension.

Single-cell analyses of the kidney and urine in hypertension

Recently, single-cell multi-omics approaches have begun to be applied to better understand the functional contribution of genetic variants uncovered by GWASs of complex diseases. Two commonly used methods to map variants to causal genes (eQTL analysis and chromatin profiling) using bulk tissue are theoretically complicated by the cellular heterogeneity of the kidney tissue, and signals from less frequent cell types may be lost in integrated bulk data sets. Data from single-cell RNA-sequencing studies provided cell-specific transcriptomic profiles that can then be used in computational deconvolution to estimate cell proportions across bulk tissue samples. Further downstream analyses (e.g., association of genotypes with gene expression) can then be corrected statistically for differences in cell proportions across samples, reducing variation between the samples and improving sensitivity for eGene detection. Two recent studies applied this strategy to kidney tissue.[7,40] One of the studies estimated ≈25% improvement in kidney eGene detection.[7]

Another advantage of single-cell multi-omics is the ability to identify potential driver cell types for different diseases. Data from single-cell RNA sequencing of the kidney helped to interpret findings from bulk tissue experiments (e.g., through mapping kidney eGenes onto specific cell types) (Figure 1c) or discover cell type–interacting eQTLs (pairs of eSNP-eGene with evidence of correlation between genotype and expression specific to certain cell types). Indeed, ≈1 in 5 of BP GWAS kidney eGenes showed cell type–specific expression in the kidney in a recent study.[40] Sheng *et al.* identified that 18% of their eQTL results are cell type specific.[7] Through combining these data with a single-cell epigenomic data set, they confirmed that cell type–interacting eQTL eSNPs showed strongest enrichment in open chromatin regions from the corresponding cell type, a reassuring consistency. Finally, they estimated driver cell types by measuring the enrichment of SBP-SNP heritability across deciles of gene expression. This analysis revealed positive correlations between SBP and distal convoluted tubules, collecting duct principal cells, and endothelial cells. Cells of collecting tubules were also enriched for BP GWAS kidney eGenes.[40]

Another elegant application of single-cell multi-omics is to infer the biological mechanisms of individual BP GWAS signals. For example, rs4292, a BP GWAS variant on chromosome 17, was confirmed as an eQTL for *ACE* in the tubule compartment. This locus occurred in open chromatin near the *ACE* transcriptional start site that contained a hepatocyte nuclear factor 4α binding motif. The *T* allele of rs4292 conferred greater hepatocyte nuclear factor 4α binding activity, and *ACE* expression was higher in tubule compartments containing this *T* allele as well.[40] This suggests that the rs4292 confers risk of increase in SBP through hepatocyte nuclear factor 4α–dependent increase in *ACE* expression. This example illustrates how a combined single-cell multi-omic approach can begin to unravel the regulatory architecture of BP GWAS variants.

Figure 2 | (continued) of *IRAK1BP1* in *cis*-eQTL analysis. The rs10943605 is also associated with kidney methylation of cg27510097 site within the promoter region of *IRAK1BP1*. IRAK1BP1 plays a role in inflammation and salt sensitivity. (**c**) The rs4582532 SNP was associated with SBP in previous GWASs. Genotype of rs4582532 is not associated with total kidney expression of *NDUFAF6* gene in *cis*-eQTL but is associated with expression of a splice isoform of *NDUFAF6* gene in the kidney. *NDUFAF6* is linked to mitochondrial function. NADH, reduced nicotinamide adenine dinucleotide.

Encouraging results from single-cell RNA sequencing of cells collected from urine suggest that this non-invasive analysis might help to gain insights into kidney parenchymal cells non-invasively. Abedini *et al.* collected clean-catch urine samples from 5 subjects.[64] From cellular pellets, they recovered most kidney parenchymal cell types, including even rare ones, such as podocytes. Interestingly, kidney cells recovered from urine showed expression of genes known as targets for antihypertensive medications (e.g., diuretics). This suggests that future single-cell multi-omic analyses can utilize non-invasive assays to better subphenotype complex diseases and capture well-established drug targets. It will be critical to develop biobanks of human urine samples that are linked to outcomes to realize this promising vision.

Epigenomics and other omics of BP and human hypertension

Methyltransferase-mediated covalent addition of methyl group to cytosines occurring primarily at CpG dinucleotides of DNA is one of the key epigenetic marks.[65] Interindividual differences in DNA methylation are at least partly determined through common genetic variants.[66,67] In fact, many SNPs control methylation of genes in their vicinity through different genetic-epigenetic interactions in *cis*.[68,69] The identity of these SNPs can be uncovered through *cis*-methylation quantitative trait locus analysis, whereby the extent of DNA methylation at any given CpG site is tested for association with genotype of SNPs mapping to its proximity. This requires samples with matching genotype and information on DNA methylation in a given tissue (e.g., from genome-wide profiles characterized by microarray or sequencing). These DNA methylation-associated SNPs are significantly enriched for alleles linked to complex disorders in GWASs.[68] Conversely, GWAS signals for complex diseases colocalize with methylation-associated SNPs.[69,70] This overlap suggests that the biological mechanisms behind GWAS association signals (90% of which map to noncoding DNA regions with no apparent biological function) may be embedded in DNA methylation. However, genome-wide evidence for the role of DNA methylation in BP regulation, human hypertension, and its kidney cophenotypes has only recently started to emerge. Most of these epigenome-wide association studies (i.e., genome-wide analyses of association between a phenotype of interest and a quantifiable epigenetic mark [e.g., DNA methylation]) were conducted using blood samples and arrays with genome-wide coverage. Indeed, large-scale analysis of DNA methylation in blood cells revealed that methylation of several CpG sites is associated with the magnitude of BP elevation[71,72] and kidney function.[73] The findings by Kato *et al.*[74] further show that at least some SNPs associated with BP in GWASs operate as methylation-associated SNPs in blood. Many epigenetic patterns are tissue and cell type specific; hence, the availability of renal tissue banks is critical to identifying epigenetic patterns clustering with kidney disease, BP, and hypertension. Our earlier studies demonstrated how genetic variants modulate the extent of renal DNA methylation within genes of importance to kidney aging.[75] The recent analysis of DNA methylation profiles of 195 kidney samples confirmed a strong contribution of common genetic variants to renal DNA methylation and demonstrated a prominent overlap between variants of importance to DNA methylation and BP.[40] Further MR studies confirmed that the extent of kidney DNA methylation shows potentially causal association with changes in BP, and at least several of the genes within this category (e.g., endothelin-1 gene [*END1*]) are already known for their role in BP regulation.[40] Others (e.g., *FES* and *IRAK1BP1*) have emerged because of their strong effect on BP across several layers of transcriptome and epigenome (Figure 2b).[40] There are several important advantages of such an integrative multi-omic approach to investigating the genetic background of a complex trait, such as BP. First, it helps to increase the rate of gene discovery and prioritize the uncovered genes based on the overall strength of evidence emerging from different omics layers[40] (i.e., genes with association documented at gene expression, DNA methylation, and other levels are apparently stronger biological contenders for further mechanistic studies). Furthermore, defining molecular mechanisms underpinning the uncovered association signals becomes feasible through connecting (e.g., DNA methylation and gene expression). Indeed, in our recent study, hypermethylation within *FES* and *IRAK1BP1* promoters was associated with a decrease in their renal expression and translated into increased BP (Figure 2b).[40] Interestingly, *FES* was 1 of 2 genes showing exome-wide significant association with hypertension in gene-wise weighted burden analysis conducted in UK Biobank.[76] This convergence of evidence makes *FES* one of the most interesting novel genes of relevance to BP and hypertension. Further studies will be warranted to elucidate the biological pathways linking *FES* to BP regulation. Availability of large data sets with other epigenetic layers, such as histone modifications (Figure 1b), is still limited. Adding the extra layers to the existing collections of human kidney tissues (with already matching information from DNA and RNA level) would further accelerate departure from a "single-molecule–single-phenotype"–based interpretation of BP GWAS signals, to chains of molecular events triggered by single DNA variants and penetrating the kidney epigenome, transcriptome, proteome, and metabolome (Figure 1d) to culminate in hypertension.

Established and new pathways and mechanisms of BP regulation emerging from kidney multi-omics

Beyond identifying genes as drivers of GWAS signals on different chromosomes, linking the molecular layers to these signals, and/or mapping potentially causal kidney genes onto cell types (Figure 1), the kidney multi-omics studies delivered important insights into biological underpinnings of hypertension and kidney disease.[7,40,59,77] Several well-known pathways of BP regulation have emerged from the convergence of BP GWASs and kidney multi-omics through shared genetic association and/or MR-derived signals of potentially causal effects on BP from their key genes. For example,

colocalization of signals for renal expression of both *AGT* and *ACE* with BP GWASs provided important genetic support for the role of intrarenal RAS in BP regulation.[7] Other well-established renal pathways of BP regulation gained additional omics support through genes with perhaps less intuitive connections to hypertension. For example, increased expression of splicing isoforms of mitogen-activated protein kinase–associated protein 1 gene (*MAPKAP1*), ubiquitin-conjugating enzyme E2E 3 gene (*UBE2E3*), and DNA methylation of *END1* in the kidney was associated with increase in BP in MR analysis integrating kidney omics with BP GWASs.[40] Both *MAPKAP1* and *UBE2E3* contribute to regulation of ENaC, a critical component of sodium reabsorption in the aldosterone-sensitive portion of the distal nephron[78] and a well-known target for BP-lowering medications (i.e., amiloride and triamterene). Indeed, *MAPKAP1* contributes to mTOR complex 2–mediated phosphorylation of serum- and glucocorticoid-inducible kinase 1, a critical activator of apical localization of ENaC and sodium reabsorption.[79,80] Acting in concert with E3 ubiquitin protein ligase gene (*NEDD4*), UBE2E3 promotes ubiquitylation of ENaC and thus regulates its rapid turnover on the surface of renal tubular epithelium.[81] Renal endothelin-1 leads to a decrease in sodium reabsorption in the collecting duct, at least in part through its inhibition of ENaC.[82] Thus, the genes contributing to key regulatory processes of ENaC expression (including phosphorylation and ubiquitylation) in the principal cells of the collecting duct received further support of potentially causal association to BP through multi-omic analysis of the kidney. Further novel pathways of established importance to other physiological processes and human diseases came to light through the application of MR to kidney multi-omics. For example, genes that encode 2 key enzymes (aminocarboxymuconate semialdehyde decarboxylase and HAAO) of kynurenine pathway responsible for catalytic breakdown of tryptophan and production of NAD^+ showed causal association with BP at the renal expression level.[40] Expressed mainly in liver and the kidney, aminocarboxymuconate semialdehyde decarboxylase limits NAD^+'s precursor (quinolinic acid) synthesis,[83] whereas HAAO converts the synthesis of quinolinic acid from 3-hydroxyanthranilic acid, promoting NAD^+ bioavailability[84]; the directionality of their causal association with BP was most consistent with the reduced production of NAD^+ in the kidney.[40] Defective NAD^+ production was postulated as a contributor to cardiovascular and kidney diseases,[84,85] and boosting its production via supplementation of its precursors or blocking its inhibitors (e.g., aminocarboxymuconate semialdehyde decarboxylase) showed potentially beneficial effects on cardiovascular system and the kidney, possibly via influence on mitochondria.[83,86]

The connection between mitochondrial homeostasis and hypertension was supported by several genes whose kidney expression, splicing, and/or DNA methylation showed causal effects on BP.[40] Functionally, these genes mapped onto (i) mitochondrial adenosine triphosphate (ATP) production pathway (e.g., ATP synthase membrane subunit 6.8PL gene [*ATP5MPL*], COX14 cytochrome *c* oxidase assembly factor gene [*COX14*], glioblastoma-amplified sequence gene [*GBAS*], *NDUFAF6* [Figure 2c], and upregulated during skeletal muscle growth 5 homolog [mouse] gene [*USMG5*]); (ii) maintenance of structural integrity of mitochondria, including mitochondrial trafficking and fusion-fission dynamics (ras homolog family member T2 gene [*RHOT2*]), or mitochondrial crista junctions (inner membrane protein, mitochondrial gene [*IMMT*]); as well as (iii) metabolic and oxidation-reduction homeostasis of mitochondria (aldo-keto reductase family 1, member B10 [aldose reductase] gene [*AKR1B10*], aldo-keto reductase family 1, member B15 gene [*AKR1B15*], 3-hydroxybutyrate dehydrogenase, type 2 gene [*BDH2*], nicotinamide nucleotide transhydrogenase gene [*NNT*], and solute carrier family 25 [mitochondrial iron transporter], member 37 gene [*SLC25A37*]).[87–91] The role of mitochondria in the development of hypertension and hypertensive kidney injury was proposed by previous studies[92,93]; these data further support the notion that genetically mediated changes in the renal DNA methylation/transcription of the autosomal genes responsible for mitochondrial function and structure may contribute to human hypertension.

Kidney multi-omics have also highlighted the importance of inflammatory and immune responses in the pathogenesis of hypertension; several kidney genes (interferon regulatory factor 5 gene [*IRF5*], tumor necrosis factor receptor-associated factor 1 gene [*TRAF1*], thioredoxin domain-containing 17 gene [*TXNDC17*], and *IRAK1BP1*) showing causal association with BP map to interferon-γ signaling, senescence, and autophagy pathways as well as tumor necrosis factor–mediated and nuclear factor-κB signaling.[94] Interferon-γ and tumor necrosis factor-α are increasingly recognized as key effector molecules in hypertensive vascular and renal injury[95]; interferons can also contribute to regulation of interrenal angiotensinogen as well as ENaC expression.[96] IRAK1BP1 has been linked to salt-sensitive hypertension by large genetic studies (Figure 2b).[97–99] Given that IRAK1BP1 functions through its effects on nuclear factor-κB as a molecular switch to bias innate immune pathways toward the resolution of inflammation,[100] its decreased expression in hypertensive kidneys may provide a mechanism for renal inflammation that can modulate hypertension (Figure 2b).[101]

Thus, a range of new biological targets mapping onto both well-recognized and emerging biological pathways and themes of relevance to BP have been identified by kidney multi-omic–based analyses. Further target studies within these pathways may uncover novel, unique diagnostic and therapeutic opportunities in hypertension and kidney injury.

Statistical and biological evidence of causality

The availability of GWAS summary statistics from international consortia and tissue multi-omics as well as large repositories of genetic information combined with rich phenotypic data has increased the popularity of MR in the area of nephrology[45] and cardiovascular medicine, with BP

and chronic kidney disease–defining traits (i.e., estimated glomerular filtration rate, albumin-to-creatinine ratio, and microalbuminuria) commonly used as "exposures" and/or "outcomes." Several important observations have emerged from these MR analyses. Increased estimated glomerular filtration rate has been linked to reduced SBP and/or DBP,[102,103] whereas lower values of SBP and/or DBP were causally associated with a decrease in urinary albumin-to-creatinine ratio, reduced risk of microalbuminuria and chronic kidney disease,[40] as well as an improvement in overall kidney health index.[53] This highlighted the importance of BP lowering in prevention of kidney complications of hypertension. Through defining contributions of anthropometric measures of obesity to estimated glomerular filtration rate, kidney health index, chronic kidney disease, and nephropathies of different etiologies (i.e., diabetic nephropathy),[53,104,105] MR reaffirmed the position of weight loss as a potentially nephroprotective strategy and characterized the extent to which hypertension and diabetes may act as mediators of these relationships.[53,105] The recent MR investigations further suggested that the causal association between body mass index and chronic kidney disease is apparent across populations of different genetic ancestry.[104,106] Other studies have applied MR to explore statistically whether changes in renal gene expression or DNA methylation are causally linked to BP, hypertension, or kidney phenotypes[40,59] and to identify potentially causal relationships across different layers of kidney omics (Figure 1e).[40,75] One of the major limitations of MR is that not all of 3 core conceptual assumptions are empirically testable,[45] so the extent to which the identified causal associations are not affected by, for example, a bias arising from not meeting these assumptions is not always feasible to verify. Different modalities of MR (e.g., that are immune to potential violations of certain MR assumptions), independent replication experiments, and sensitivity analyses (e.g., with an exclusion of certain genetic instruments) are commonly used to confirm the robustness of the initial MR findings. However, even with all core assumptions of MR theoretically satisfied, the ultimate insights of whether/how genes lead to a disease require further functional studies using cellular experiments (*in vitro*) and/or animal studies (*in vivo*) (Figure 1f).[107,108]

One of the most powerful approaches used to examine the biological significance of findings from GWASs is genome editing via *in vitro* CRISPR/Cas9 system (Figure 1f).[107] The targeted editing (e.g., deletion of a region containing a GWAS SNP or specific allelic variants of a GWAS SNP) in either cellular or animal models allows assigning the differences in captured molecular or phenotypic readouts to the edited region/SNP of interest.[107] Such functional experiments using CRISPR/Cas9 system have started to emerge in post-GWAS analyses of cardiovascular and kidney diseases.[7,107,109,110] For example, CRISPR-based edition of the regulatory regions containing SNPs implicated through prior GWAS analyses of BP and kidney function confirmed how deletion of these regions translated into the changes in expression of kidney eGenes, such as fibroblast growth factor 5 gene (*FGF5*), *ACE*,[7] and Dachshund homolog 1 (*DACH1*) *in vitro*.[110] Single-base edition through CRISPR/Cas9 system can be technically challenging,[109] but successful generation of isogenic stem cell lines (reflective of, for example, 2 homozygous genotypes of a GWAS SNP) is a powerful demonstration of the functional effects of the individual SNP. Such CRISPR/Cas9-based edition of a biallelic variant associated with hypertension and other cardiovascular diseases (*rs9349379*) proved the regulatory effect of this specific SNP on *EDN1* expression.[109] CRISPR/Cas9 system was also shown to effectively methylate/demethylate CpG islands, gene promoters, and enhancers in a targeted manner.[111] Thus, DNA methylation editing may be also exploited to examine the biological significance of uncovered kidney CpG sites and provide further mechanistic insights into their regulatory role.

Kidney omics and hypertension: examples of clinical implications

BP GWASs and their downstream kidney multi-omics studies highlighted several promising translational routes to clinical practice. One of them is via polygenic risk scores. Polygenic risk scores aggregate effects of allelic variants from SNPs across the genome to identify individuals at higher risk of disease.[112,113] In one of the largest published GWASs of BP to date,[30] individuals in the top decile of the polygenic risk score distribution had 12.9–mm Hg higher mean SBP than those in the bottom decile, a 3.3-fold increased risk of hypertension, and ≈1.5-fold increased risk of incident cardiovascular disease, myocardial infarction, and stroke. This supports the possibility of targeted lifestyle intervention measures in young adults to control BP and reduce the risk of developing cardiovascular disease in later life for those at genetic risk of increased BP.

One of the most practical implications of uncovering the causes of monogenic forms of hypertension was personalization of antihypertensive treatment (i.e., tailoring the choice of BP-lowering agents based on knowledge of an individual's genetic make-up).[114] Indeed, by blocking upregulated ENaCs on the surface of tubular epithelium, amiloride was determined as the drug of choice in Liddle syndrome, whereas thiazides were found to correct metabolic abnormalities and lower BP in those with Gordon syndrome.[114] Such a level of precision medicine is not yet feasible for a vast majority of hypertensive patients, but kidney omics are making contributions to drug discovery and "rediscovery" in hypertension. Indeed, kidney transcriptomics and epigenomics combined with genetics and the newest statistical strategies provided strong support for existing medications known for their BP-lowering potential, such as ACE inhibitors, minoxidil, diazoxide, nitrates, riociguat, and amiloride.[7,40,53] Kidney omics also uncovered new connections within established pathways of BP regulation (e.g., by demonstrating cell type–specific transcriptional regulators of *ACE*[7] and uncovering new coding and noncoding partner elements within the network of

kidney ACE2[44] and renin[6]). These deliveries of kidney omics may stimulate research into new strategies of interfering with RAS to achieve therapeutic outcomes with perhaps less off-target effects.[115] Apart from these insights, the rediscovery of RAS components as BP regulators by kidney multi-omics (Figure 1g) demonstrates robustness of these strategies to uncover other genes and molecules of importance to BP and hypertension. However, the key translational delivery methods emerging from kidney omics are genes and pathways with an established role in human physiology but underexplored role/connections to BP regulation; many of these molecules will be targets for already existing, well-known therapeutics. Indeed, new exciting drug repurposing opportunities for hypertension have started to emerge from kidney multi-omics (e.g., topiramate [a medication used in management of epilepsy and migraine] was implicated as a drug with a BP-lowering potential).[40] Such sets of genes are perhaps the most promising candidates for new antihypertensive medications via drug repositioning.

Taken together, these data highlight exciting opportunities to stratify patients according to polygenic risk score to identify those at greatest risk of hypertension and show the promise of kidney omics to inform the drug discovery for hypertension and hypertension-mediated kidney damage.

Summary

Publicly available access to data from large-scale GWASs and biobanks has led to a rapid pace in discovery of genetic associations with many complex traits and diseases, including BP and hypertension. Exploration of the kidney transcriptome and epigenome using advances in statistics and bioinformatics yielded the first insights into kidney genes, mRNA isoforms, and epigenetic signatures underpinning the statistical associations from BP GWASs. Many kidney genes showed causal association to BP at several molecular layers; such genes are the priority for further *in vitro* and *in vivo* studies to elucidate their functional relevance to BP regulation and hypertension (Figure 2). Although large RNA-sequencing–derived collections of human kidney are currently best available resources for quantifying the abundance of thousands of renal genes simultaneously with a high level of precision, the development of similar data sets of kidney tissue characterized at protein level is of utmost importance given the variable level of concordance between mRNAs and proteins and the outputs from eQTL and protein quantitative trait locus analyses.[116] Seeking associations between abundance of a given protein in a cell/tissue and SNPs in the *cis* window, protein quantitative trait locus studies have already started to emerge from collections of human plasma/serum characterized by, for example, the slow-off rate modified aptamer (SOMAmer) platform (Figure 1d).[116] Through convergence with GWASs, such data sets are beginning to gain initial insights into the genetic background of kidney health and disease.[117] Integration of genomics, transcriptomics, and proteomics holds the promise of accelerating progress in drug development in precision medicine.[118]

Medical advances will further depend on defining genomic and environmental interactions that determine the signature pathogenic cell and developmental stage-specific expression patterns. Such a comprehensive approach is essential to address clinically relevant issues, such as predisposition to hypertension on high-salt diet or exposure to nonsteroidal anti-inflammatory drugs and to glomerular hypertension and associated damage in chronic kidney disease.

Finally, concerted efforts are necessary to create new or enrich the existing tissue collections through securing samples from patients of diverse ancestry and origin. The underrepresentation of patients of non-White European ancestry is of course not exclusive to human tissue data sets and is apparent across many genetic studies and publicly available resources.[119] Increasing diversity in GWASs and post-GWAS explorations is necessary for full understanding of molecular consequences of disease-associated alleles in the global human population.

DISCLOSURE

JMMH is a full-time employee of Novo Nordisk Ltd. SD holds a patent, "Mitochondria-Targeted Isoketal/Isolevuglandin Scavengers" (international application number PCT/US2020/015197). All the other authors declared no competing interests.

ACKNOWLEDGMENTS

This work was supported by British Heart Foundation grants (PG/17/35/33001 and PG/19/16/34270) and Kidney Research UK grants (RP_017_20180302 and RP_013_20190305) to MT; and National Institutes of Health (NIH) grants (R01 DK117445-01A1 and R01 MD012765) to NF and APM. APM acknowledges support from Versus Arthritis (grant reference 21754). TJG is funded by European Research Council InflammaTENSION; ERC-CoG-726318 and CVD ERA-CVD (PLAQUEFIGHT; 01KL1808; NCBiR, Poland, and Brain-Gut). SD is funded by NIH grants (R01 HL144943 and R01 HL157583).

REFERENCES

1. Zhou B, Perel P, Mensah GA, et al. Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. *Nat Rev Cardiol*. 2021;18:785–802.
2. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396:1223–1249.
3. Guyton AC, Coleman TG, Cowley AV, et al. Arterial pressure regulation: overriding dominance of the kidneys in long-term regulation and in hypertension. *Am J Med*. 1972;52:584–594.
4. Hall JE, Brands MW, Henegar JR. Angiotensin II and long-term arterial pressure regulation: the overriding dominance of the kidney. *J Am Soc Nephrol*. 1999;10(suppl 12):S258–S265.
5. Yang T, Xu C. Physiology and pathophysiology of the intrarenal renin-angiotensin system: an update. *J Am Soc Nephrol*. 2017;28:1040–1049.
6. Marques FZ, Romaine SP, Denniff M, et al. Signatures of miR-181a on the renal transcriptome and blood pressure. *Mol Med*. 2015;21:739–748.
7. Sheng X, Guan Y, Ma Z, et al. Mapping the genetic architecture of human traits to cell types in the kidney identifies mechanisms of disease and potential treatments. *Nat Genet*. 2021;53:1322–1333.
8. Lifton RP, Gharavi AG, Geller DS. Molecular mechanisms of human hypertension. *Cell*. 2001;104:545–556.
9. Tobin MD, Tomaszewski M, Braund PS, et al. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. *Hypertension*. 2008;51:1658–1664.
10. Busst CJ, Bloomer LD, Scurrah KJ, et al. The epithelial sodium channel gamma-subunit gene and blood pressure: family based association, renal gene expression, and physiological analyses. *Hypertension*. 2011;58:1073–1078.

11. Figtree GA, Broadfoot K, Casadei B, et al. A call to action for new global approaches to cardiovascular disease drug solutions. *Eur Heart J*. 2021;42:1464–1475.
12. Raina R, Krishnappa V, Das A, et al. Overview of monogenic or Mendelian forms of hypertension. *Front Pediatr*. 2019;7:263.
13. Havlik RJ, Garrison RJ, Feinleib M, et al. Blood pressure aggregation in families. *Am J Epidemiol*. 1979;110:304–312.
14. Harrap SB, Stebbing M, Hopper JL, et al. Familial patterns of covariation for cardiovascular risk factors in adults: the Victorian Family Heart Study. *Am J Epidemiol*. 2000;152:704–715.
15. Wray NR, Visscher PM. Estimating trait heritability. *Nat Educ*. 2008;1:29.
16. Kolifarhood G, Daneshpour M, Hadaegh F, et al. Heritability of blood pressure traits in diverse populations: a systematic review and meta-analysis. *J Hum Hypertens*. 2019;33:775–785.
17. Parati G, Ochoa JE, Bilo G. Moving beyond office blood pressure to achieve a personalized and more precise hypertension management. *Hypertension*. 2017;70:e20–e31.
18. Tomaszewski M, Debiec R, Braund PS, et al. Genetic architecture of ambulatory blood pressure in the general population: insights from cardiovascular gene-centric array. *Hypertension*. 2010;56:1069–1076.
19. Swales J, ed. *Platt Versus Pickering: An Episode in Recent Medical History*. Vol 8. Keynes Press (British Medical Association); 1985.
20. Levy D, Ehret GB, Rice K, et al. Genome-wide association study of blood pressure and hypertension. *Nat Genet*. 2009;41:677–687.
21. Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet*. 2009;41:527–534.
22. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature*. 2011;478:103–109.
23. Wain LV, Verwoert GC, O'Reilly PF, et al. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. *Nat Genet*. 2011;43:1005–1011.
24. Kato N, Takeuchi F, Tabara Y, et al. Meta-analysis of genome-wide association studies identifies common variants associated with blood pressure variation in east Asians. *Nat Genet*. 2011;43:531–538.
25. Franceschini N, Fox E, Zhang Z, et al. Genome-wide association analysis of blood-pressure traits in African-ancestry individuals reveals common associated genes in African and non-African populations. *Am J Hum Genet*. 2013;93:545–554.
26. Hoffmann TJ, Ehret GB, Nandakumar P, et al. Genome-wide association analyses using electronic health records identify new loci influencing blood pressure variation. *Nat Genet*. 2017;49:54–64.
27. Wain LV, Vaez A, Jansen R, et al. Novel blood pressure locus and gene discovery using genome-wide association study and expression data sets from blood and the kidney. *Hypertension*. 2017;70:e4–e19.
28. Liang J, Le TH, Edwards DRV, et al. Single-trait and multi-trait genome-wide association analyses identify novel loci for blood pressure in African-ancestry populations. *PLoS Genet*. 2017;13:e1006728.
29. Warren HR, Evangelou E, Cabrera CP, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nat Genet*. 2017;49:403–415.
30. Evangelou E, Warren HR, Mosen-Ansorena D, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet*. 2018;50:1412–1425.
31. Giri A, Hellwege JN, Keaton JM, et al. Trans-ethnic association study of blood pressure determinants in over 750,000 individuals. *Nat Genet*. 2019;51:51–62.
32. Wojcik GL, Graff M, Nishimura KK, et al. Genetic analyses of diverse populations improves discovery for complex traits. *Nature*. 2019;570:514–518.
33. Sung YJ, Winkler TW, de Las Fuentes L, et al. A large-scale multi-ancestry genome-wide study accounting for smoking behavior identifies multiple significant loci for blood pressure. *Am J Hum Genet*. 2018;102:375–400.
34. Kanai M, Akiyama M, Takahashi A, et al. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat Genet*. 2018;50:390–400.
35. Surendran P, Drenos F, Young R, et al. Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. *Nat Genet*. 2016;48:1151–1161.
36. Liu C, Kraja AT, Smith JA, et al. Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. *Nat Genet*. 2016;48:1162–1170.
37. Surendran P, Feofanova EV, Lahrouchi N, et al. Discovery of rare variants associated with blood pressure regulation through meta-analysis of 1.3 million individuals. *Nat Genet*. 2020;52:1314–1332.
38. Backman JD, Li AH, Marcketta A, et al. Exome sequencing and analysis of 454,787 UK Biobank participants. *Nature*. 2021;599:628–634.
39. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. 2013;45:580–585.
40. Eales JM, Jiang X, Xu X, et al. Uncovering genetic mechanisms of hypertension through multi-omic analysis of the kidney. *Nat Genet*. 2021;53:630–637.
41. Gillies CE, Putler R, Menon R, et al. An eQTL landscape of kidney tissue in human nephrotic syndrome. *Am J Hum Genet*. 2018;103:232–244.
42. Tomaszewski M, Charchar FJ, Nelson CP. Pathway analysis shows association between FGFBP1 and hypertension. *J Am Soc Nephrol*. 2011;22:947–955.
43. Tomaszewski M, Eales J, Denniff M, et al. Renal mechanisms of association between fibroblast growth factor 1 and blood pressure. *J Am Soc Nephrol*. 2015;26:3151–3160.
44. Jiang X, Eales JM, Scannali D, et al. Hypertension and renin-angiotensin system blockers are not associated with expression of angiotensin-converting enzyme 2 (ACE2) in the kidney. *Eur Heart J*. 2020;41:4580–4588.
45. Sekula P, Del Greco MF, Pattaro C, et al. Mendelian randomization as an approach to assess causality using observational data. *J Am Soc Nephrol*. 2016;27:3253–3265.
46. Gil RB, Ortiz A, Sanchez-Nino MD, et al. Increased urinary osmolyte excretion indicates chronic kidney disease severity and progression rate. *Nephrol Dial Transplant*. 2018;33:2156–2164.
47. Gotthardt K, Lokaj M, Koerner C, et al. A G-protein activation cascade from Arl13B to Arl3 and implications for ciliary targeting of lipidated proteins. *Elife*. 2015;4:e11859.
48. Thompson CD, Matta B, Barnes BJ. Therapeutic targeting of IRFs: pathway-dependence or structure-based? *Front Immunol*. 2018;9:2622.
49. Shi H, Enriquez A, Rapadas M, et al. NAD deficiency, congenital malformations, and niacin supplementation. *N Engl J Med*. 2017;377:544–552.
50. Lorenzen JM, Thum T. Long noncoding RNAs in kidney and cardiovascular diseases. *Nat Rev Nephrol*. 2016;12:360–373.
51. Carlstrom M. Nitric oxide signalling in kidney regulation and cardiometabolic health. *Nat Rev Nephrol*. 2021;17:575–590.
52. Emdin CA, Khera AV, Klarin D, et al. Phenotypic consequences of a genetic predisposition to enhanced nitric oxide signaling. *Circulation*. 2018;137:222–232.
53. Xu X, Eales JM, Jiang X, et al. Contributions of obesity to kidney health and disease – insights from Mendelian randomisation and the human kidney transcriptomics. *Cardiovasc Res*. Published online December 10, 2021. https://doi.org/10.1093/cvr/cvab357
54. Hughey RP, Bruns JB, Kinlough CL, et al. Epithelial sodium channels are activated by furin-dependent proteolysis. *J Biol Chem*. 2004;279:18111–18114.
55. Semenov AG, Tamm NN, Seferian KR, et al. Processing of pro-B-type natriuretic peptide: furin and corin as candidate convertases. *Clin Chem*. 2010;56:1166–1176.
56. Dubois CM, Blanchette F, Laprise MH, et al. Evidence that furin is an authentic transforming growth factor-beta1-converting enzyme. *Am J Pathol*. 2001;158:305–316.
57. Van der Wel T, Hilhorst R, Den Dulk H, et al. Chemical genetics strategy to profile kinase target engagement reveals role of FES in neutrophil phagocytosis. *Nat Commun*. 2020;11:3216.
58. Liu B, Pjanic M, Wang T, et al. Genetic regulatory mechanisms of smooth muscle cells map to coronary artery disease risk loci. *Am J Hum Genet*. 2018;103:377–388.
59. Xu X, Eales JM, Akbarov A, et al. Molecular insights into genome-wide association studies of chronic kidney disease-defining traits. *Nat Commun*. 2018;9:4800.
60. Marques FZ, Booth SA, Charchar FJ. The emerging role of non-coding RNA in essential hypertension and blood pressure regulation. *J Hum Hypertens*. 2015;29:459–467.
61. Romaine SP, Tomaszewski M, Condorelli G, et al. MicroRNAs in cardiovascular disease: an introduction for clinicians. *Heart*. 2015;101:921–928.

62. Romaine SP, Charchar FJ, Samani NJ, et al. Circulating microRNAs and hypertension–from new insights into blood pressure regulation to biomarkers of cardiovascular risk. *Curr Opin Pharmacol*. 2016;27:1–7.
63. Prestes PR, Maier MC, Woods BA, et al. A guide to the short, long and circular RNAs in hypertension and cardiovascular disease. *Int J Mol Sci*. 2020;21:3666.
64. Abedini A, Zhu YO, Chatterjee S, et al. Urinary single-cell profiling captures the cellular diversity of the kidney. *J Am Soc Nephrol*. 2021;32:614–627.
65. Zhu H, Wang G, Qian J. Transcription factors as readers and effectors of DNA methylation. *Nat Rev Genet*. 2016;17:551–565.
66. Gamazon ER, Badner JA, Cheng L, et al. Enrichment of *cis*-regulatory gene expression SNPs and methylation quantitative trait loci among bipolar disorder susceptibility variants. *Mol Psychiatry*. 2013;18:340–346.
67. Voisin S, Almen MS, Zheleznyakova GY, et al. Many obesity-associated SNPs strongly associate with DNA methylation changes at proximal promoters and enhancers. *Genome Med*. 2015;7:103.
68. Smith AK, Kilaru V, Kocak M, et al. Methylation quantitative trait loci (meQTLs) are consistently detected across ancestry, developmental stage, and tissue type. *BMC Genomics*. 2014;15:145.
69. Do C, Shearer A, Suzuki M, et al. Genetic-epigenetic interactions in *cis*: a major focus in the post-GWAS era. *Genome Biol*. 2017;18:120.
70. Do C, Lang CF, Lin J, et al. Mechanisms and disease associations of haplotype-dependent allele-specific DNA methylation. *Am J Hum Genet*. 2016;98:934–955.
71. Richard MA, Huan T, Ligthart S, et al. DNA methylation analysis identifies loci for blood pressure regulation. *Am J Hum Genet*. 2017;101:888–902.
72. Huang Y, Ollikainen M, Muniandy M, et al. Identification, heritability, and relation with gene expression of novel DNA methylation loci for blood pressure. *Hypertension*. 2020;76:195–205.
73. Breeze CE, Batorsky A, Lee MK, et al. Epigenome-wide association study of kidney function identifies trans-ethnic and ethnic-specific loci. *Genome Med*. 2021;13:74.
74. Kato N, Loh M, Takeuchi F, et al. Trans-ancestry genome-wide association study identifies 12 genetic loci influencing blood pressure and implicates a role for DNA methylation. *Nat Genet*. 2015;47:1282–1293.
75. Rowland J, Akbarov A, Eales J, et al. Uncovering genetic mechanisms of kidney aging through transcriptomics, genomics, and epigenomics. *Kidney Int*. 2019;95:624–635.
76. Curtis D. Analysis of 200,000 exome-sequenced UK Biobank subjects implicates genes involved in increased and decreased risk of hypertension. *Pulse (Basel)*. 2021;9:17–29.
77. Qiu C, Huang S, Park J, et al. Renal compartment-specific genetic variation analyses identify new pathways in chronic kidney disease. *Nat Med*. 2018;24:1721–1731.
78. Lang F, Pearce D. Regulation of the epithelial Na+ channel by the mTORC2/SGK1 pathway. *Nephrol Dial Transplant*. 2016;31:200–205.
79. Lu M, Wang J, Ives HE, et al. mSIN1 protein mediates SGK1 protein interaction with mTORC2 protein complex and is required for selective activation of the epithelial sodium channel. *J Biol Chem*. 2011;286:30647–30654.
80. Sorensen MV, Saha B, Jensen IS, et al. Potassium acts through mTOR to regulate its own secretion. *JCI Insight*. 2019;5:e126910.
81. Staub O, Verrey F. Impact of Nedd4 proteins and serum and glucocorticoid-induced kinases on epithelial Na+ transport in the distal nephron. *J Am Soc Nephrol*. 2005;16:3167–3174.
82. Lynch IJ, Welch AK, Kohan DE, et al. Endothelin-1 inhibits sodium reabsorption by ET(A) and ET(B) receptors in the mouse cortical collecting duct. *Am J Physiol Renal Physiol*. 2013;305:F568–F573.
83. Katsyuba E, Mottis A, Zietak M, et al. *De novo* NAD(+) synthesis enhances mitochondrial function and improves health. *Nature*. 2018;563:354–359.
84. Badawy AA. Kynurenine pathway of tryptophan metabolism: regulatory and functional aspects. *Int J Tryptophan Res*. 2017;10:1178646917691938.
85. Zakrocka I, Zaluska W. Kynurenine pathway in kidney diseases. *Pharmacol Rep*. 2022;74:27–39.
86. Martens CR, Denman BA, Mazzo MR, et al. Chronic nicotinamide riboside supplementation is well-tolerated and elevates NAD(+) in healthy middle-aged and older adults. *Nat Commun*. 2018;9:1286.
87. Song J, Pfanner N, Becker T. Assembling the mitochondrial ATP synthase. *Proc Natl Acad Sci U S A*. 2018;115:2850–2852.
88. Vercellino I, Sazanov LA. The assembly, regulation and function of the mitochondrial respiratory chain. *Nat Rev Mol Cell Biol*. 2022;23:141–161.
89. Tang C, Cai J, Yin XM, et al. Mitochondrial quality control in kidney injury and repair. *Nat Rev Nephrol*. 2021;17:299–318.
90. Leskov I, Neville A, Shen X, et al. Nicotinamide nucleotide transhydrogenase activity impacts mitochondrial redox balance and the development of hypertension in mice. *J Am Soc Hypertens*. 2017;11:110–121.
91. Soledad RB, Charles S, Samarjit D. The secret messages between mitochondria and nucleus in muscle cell biology. *Arch Biochem Biophys*. 2019;666:52–62.
92. Dikalov SI, Dikalova AE. Crosstalk between mitochondrial hyperacetylation and oxidative stress in vascular dysfunction and hypertension. *Antioxid Redox Signal*. 2019;31:710–721.
93. Eirin A, Lerman A, Lerman LO. Enhancing mitochondrial health to treat hypertension. *Curr Hypertens Rep*. 2018;20:89.
94. Pan XX, Wu F, Chen XH, et al. T-cell senescence accelerates angiotensin II-induced target organ damage. *Cardiovasc Res*. 2021;117:271–283.
95. Higaki A, Mahmoud AUM, Paradis P, et al. Role of interleukin-23/interleukin-17 axis in T-cell-mediated actions in hypertension. *Cardiovasc Res*. 2021;117:1274–1283.
96. Satou R, Miyata K, Gonzalez-Villalobos RA, et al. Interferon-gamma biphasically regulates angiotensinogen expression via a JAK-STAT pathway and suppressor of cytokine signaling 1 (SOCS1) in renal proximal tubular cells. *FASEB J*. 2012;26:1821–1830.
97. Guzik TJ, Harrison DG. Endothelial NF-kappaB as a mediator of kidney damage: the missing link between systemic vascular and renal disease? *Circ Res*. 2007;101:227–229.
98. Razavi MA, Bazzano LA, Nierenberg J, et al. Advances in genomics research of blood pressure responses to dietary sodium and potassium intakes. *Hypertension*. 2021;78:4–15.
99. Murray EC, Nosalski R, MacRitchie N, et al. Therapeutic targeting of inflammation in hypertension: from novel mechanisms to translational perspective. *Cardiovasc Res*. 2021;117:2589–2609.
100. Conner JR, Smirnova II, Moseman AP, Poltorak A. IRAK1BP1 inhibits inflammation by promoting nuclear translocation of NF-kappaB p50. *Proc Natl Acad Sci U S A*. 2010;107:11477–11482.
101. Drummond GR, Vinh A, Guzik TJ, Sobey CG. Immune mechanisms of hypertension. *Nat Rev Immunol*. 2019;19:517–532.
102. Yu Z, Coresh J, Qi G, et al. A bidirectional Mendelian randomization study supports causal effects of kidney function on blood pressure. *Kidney Int*. 2020;98:708–716.
103. Morris AP, Thu HL, Wu H, et al. Trans-ethnic kidney function association study reveals putative causal genes and effects on kidney-specific disease aetiologies. *Nat Commun*. 2019;10:29.
104. Zheng J, Zhang Y, Rasheed H, et al. Trans-ethnic Mendelian-randomization study reveals causal relationships between cardiometabolic factors and chronic kidney disease. *Int J Epidemiol*. 2021;50:1995–2010.
105. Zhu P, Herrington WG, Haynes R, et al. Conventional and genetic evidence on the association between adiposity and CKD. *J Am Soc Nephrol*. 2021;32:127–137.
106. Ye C, Kong L, Zhao Z, et al. Causal associations of obesity with chronic kidney disease and arterial stiffness: a Mendelian randomization study. *J Clin Endocrinol Metab*. 2022;107:e825–e835.
107. Lin J, Musunuru K. From genotype to phenotype: a primer on the functional follow-up of genome-wide association studies in cardiovascular disease. *Circ Genom Precis Med*. 2018;11:e001946.
108. Claussnitzer M, Susztak K. Gaining insight into metabolic diseases from human genetic discoveries. *Trends Genet*. 2021;37:1081–1094.
109. Gupta RM, Hadaya J, Trehan A, et al. A genetic variant associated with five vascular diseases is a distal regulator of endothelin-1 gene expression. *Cell*. 2017;170:522–533.e15.

110. Doke T, Huang S, Qiu C, et al. Transcriptome-wide association analysis identifies DACH1 as a kidney disease risk gene that contributes to fibrosis. *J Clin Invest*. 2021;131:e141801.
111. Pulecio J, Verma N, Mejía-Ramírez E, et al. CRISPR/Cas9-based engineering of the epigenome. *Cell Stem Cell*. 2017;21:431–447.
112. Polygenic Risk Score Task Force of the International Common Disease Alliance. Responsible use of polygenic risk scores in the clinic: potential benefits, risks and gaps. *Nat Med*. 2021;27:1876–1884.
113. Ding Y, Hou K, Burch KS, et al. Large uncertainty in individual polygenic risk score estimation impacts PRS-based risk stratification. *Nat Genet*. 2022;54:30–39.
114. Tomaszewski M, Zimmerli L, Charchar FJ, et al. Genetic information in the diagnosis and treatment of hypertension. *Curr Hypertens Rep*. 2006;8:309–316.
115. Morgan ES, Tami Y, Hu K, et al. Antisense inhibition of angiotensinogen with IONIS-AGT-L_{Rx}: results of phase 1 and phase 2 studies. *J Am Coll Cardiol Basic Trans Science*. 2021;6:485–496.
116. Gudjonsson A, Valborg Gudmundsdottir V, Axelsson GT, et al. A genome-wide association study of serum proteins reveals shared loci with common diseases. *Nat Commun*. 2022;13:480.
117. Matías-García P, Wilson R, Guo Q, et al. Plasma proteomics of renal function: a trans-ethnic meta-analysis and Mendelian randomization study. *J Am Soc Nephrol*. 2021;32:1747–1763.
118. Schunkert H, Mayr M. Linking genetics and proteomics: gene-protein associations built on diversity. *Circulation*. 2022;145:371–374.
119. Uffelmann E, Huang QQ, Munung NS, et al. Genome-wide association studies. *Nat Rev Methods Primers*. 2021;59.