REVIEW

Novel biomarkers and emerging tools to identify causal molecular pathways in hypertension and associated cardiovascular diseases

Ewelina Józefczuk[1, 2], Tomasz J Guzik[1–3], Mateusz Siedliński[1, 2]

[1]Department of Internal and Agricultural Medicine, Jagiellonian University Medical College, Kraków, Poland
[2]Center for Medical Genomics OMICRON, Jagiellonian University Medical College, Kraków, Poland
[3]Center for Cardiovascular Sciences; Queen's Medical Research Institute; University of Edinburgh, University of Edinburgh, Edinburgh, United Kingdom

Correspondence to:
Mateusz Siedliński, MD, PhD, Department of Internal and Agricultural Medicine, Jagiellonian University Medical College, Skarbowa 1, 31–121 Kraków, Poland, phone: +48 12 633 00 03, e-mail: mateusz.siedlinski@uj.edu.pl

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ABSTRACT

Hypertension (HT) is a modifiable risk factor for life-threatening cardiovascular diseases (CVDs) including coronary artery disease, heart failure, or stroke. Despite significant progress in understanding the pathophysiological mechanisms of the disease, the molecular pathways targeted by HT treatment remain largely unchanged. This warrants the need for finding novel biomarkers, which are causally related to persistent high blood pressure (BP) and may be pharmacologically targeted. Analytical output derived from large-scale biobanks, containing high-throughput genetic and biochemical data, such as OLINK and SomaScan-based proteomics or Nuclear Magnetic Resonance-based metabolomics, as well as novel analytical tools including the Mendelian randomization (MR) approach, enabling genetic causal inference, may create new treatment opportunities for HT and related CVDs. MR analysis may constitute additional evidence for observational studies and facilitate selection of drug targets for clinical testing and has been already used to nominate potentially causal biomarkers for HT and CVDs such as circulating glycine, branched-chain amino acids, lipoprotein(a), insulin-like growth factor 1, or fibronectin 1. Using the MR framework, genetic proxies for targets of already known drugs, such as statins, PCSK9, and ACE inhibitors, may additionally be informative about potential side effects and eventually contribute to more personalized medicine. Finally, genetic causal inference may disentangle independent direct effects of correlated traits such as lipid classes or markers of inflammation on cardiovascular clinical outcomes such as atherosclerosis and HT. While several novel HT-targeting drugs are currently under clinical investigation (e.g. brain renin-angiotensin-aldosterone system inhibitors or endothelin-1 receptor antagonists), analysis of high-throughput proteomic and metabolomic data from well-powered studies may deliver novel druggable molecular targets for HT and associated CVDs.

Key words: biomarker, blood pressure, cardiovascular disease, hypertension, Mendelian randomization

INTRODUCTION

Systemic arterial hypertension (HT) remains one of the main healthcare problems worldwide, being insufficiently diagnosed, prognosed and treated [1]. The global prevalence of HT and the number of HT-related deaths increase, indicating inefficiency of the overall disease control system [2, 3]. Despite significant advancement that has been made in the understanding of the molecular mechanisms orchestrating HT pathophysiology, currently used antihypertensive drugs are still based on molecular targets developed decades ago, which are of limited effectiveness, have to be administered in combination, and may cause unwanted side effects [4–6]. However, the dynamic development of genomic studies on HT and associated cardiovascular diseases (CVDs) as well as novel analytical tools, including the Mendelian randomization (MR) approach that may deliver partial evidence on the cause--effect relation between biomarkers and clini-

cal outcomes, holds a promise for the breakthrough in the management of HT and CVDs.

Essential HT is a multifaceted and multifactorial disorder, caused by dysfunction of multiple physiological systems, which was pointed out as early as 1949 by Irvine H Page in the so-called "mosaic theory" [7, 8]. HT is caused by both genetic and various other, at least partially heritable factors, for instance, the level of body mass index, smoking habits, or alcohol intake frequency. All the genetic and environmental risk factors may affect the level of blood pressure (BP) by targeting many specific biomarkers such as proteins or metabolites in the bloodstream or target organs. Previous studies on HT pathophysiology have led to a discovery of a great variety of molecular pathways involved in the development and progression of HT. Established biomarkers with a well-documented role in HT, such as endothelin-1 (ET-1), studied in long-term and extensive preclinical and observational studies, are now under clinical investigation as potential diagnostic or therapeutic targets. However, recent progress made in the understanding of HT genetics by well-designed and large-scale studies utilizing high-throughput genomic, proteomic, and metabolomic methods, markedly accelerates discoveries of relevant biomarkers. The identification of biomarkers that are members of causal pathways regulating BP is of high clinical relevance and may help to reduce the global burden of HT and associated CVDs [9].

SEARCHING FOR BIOMARKERS OF HT AND ASSOCIATED CVDS

The translation route from a newly identified biomarker to a druggable target is long and complex. Before the genome-wide association studies (GWASs) era, identification of new components of the molecular BP machinery was limited to testing predefined candidates, selected on the basis of previous observations. The rapid development of GWASs significantly accelerated HT research, as it enabled scanning of the whole genome to search for genetic markers not considered *a priori* as relevant for BP control [10]. Combining GWAS technology with large population studies has led to identification of more than 1000 genetic loci associated with BP, creating new opportunities for HT treatment [11, 12]. Extensive post-GWAS studies are now being conducted in animal experimental models with translation to human-derived biological material to evaluate functional and mechanistic characteristics of newly discovered biomarkers. Importantly, other "omic" strategies (e.g. transcriptomics, proteomics, lipidomics, metabolomics) are being continuously developed and mutually combined [13, 14].

Observational and predominantly cross-sectional studies have associated levels of numerous biomarkers with HT or CVDs [15, 16]. However, by definition, such studies were often unable to unravel whether the observed association was of causal nature or whether the change in biomarker level was a consequence of disease progression or simple confounding. This has been recently presented by Porcu et al. [17] in a study revealing that differences in the level of blood mRNA transcripts between healthy and diseased subjects may more likely reflect the effect of the disease on mRNA transcript level rather than the causal effect of gene expression on the disease outcome. Such phenomenon has been also observed by the SCALLOP consortium reporting that the level of all 90 investigated plasma proteins was genetically altered by at least 1 genetically complex disease [18].

MENDELIAN RANDOMIZATION AS AN APPROACH TO ASSESS POTENTIAL CAUSALITY

In order to find novel molecular targets for HT and associated CVDs, which could be selected for further clinical testing in interventional studies, there is a need to investigate the possibility of reverse causation and to control confounding. The most substantial evidence of causality is given by randomized controlled trials (RCTs), however, for a number of ethical and technical reasons, it is rarely justified or feasible to design and perform this type of study on the basis of available premises. The barriers in translation of preclinical HT science into clinical practice were reviewed lately by Sigmund et al. [13]. Nevertheless, the availability of well-designed and large-scale GWASs creates a unique opportunity to verify previously identified observational associations in terms of causality and to search for novel biomarkers causal for BP level using the Mendelian randomization (MR) approach [19, 20] (Figure 1). This approach utilizes random segregation of alleles at gametogenesis and uses inherited genetic variations (e.g. single nucleotide polymorphisms [SNPs]) as instrumental variables (IVs) to estimate the effect of modifiable exposure, such as the level of a biomarker, on the outcome, e.g. coronary artery disease (CAD) or the level of BP. Three key assumptions of the MR analysis concern the association between IVs and exposure (the relevance assumption), lack of horizontal pleiotropy (the exclusion restriction assumption), and lack of unmeasured confounders of the association between IVs and the outcome (the independence assumption) [19] (Figure 1). When these assumptions are met, the causal MR estimate should not be affected by confounding, which is encountered in observational studies and may bias their results. Currently, RCTs remain the best approach to test causal directions between various traits and clinical parameters [19, 21], and genetics-based tests, such as MR, can provide evidence in addition to that obtained in experimental and observational studies [19]. MR-based approaches may be of particular relevance when interventional studies are difficult or even impossible to perform, and as an additional evidence for observational association before conducting expensive RCTs. For example, while various lipid types have been extensively studied as risk factors for CAD, MR helped to unravel that apolipoprotein B predominantly accounts for the association of lipoproteins such as LDL-C

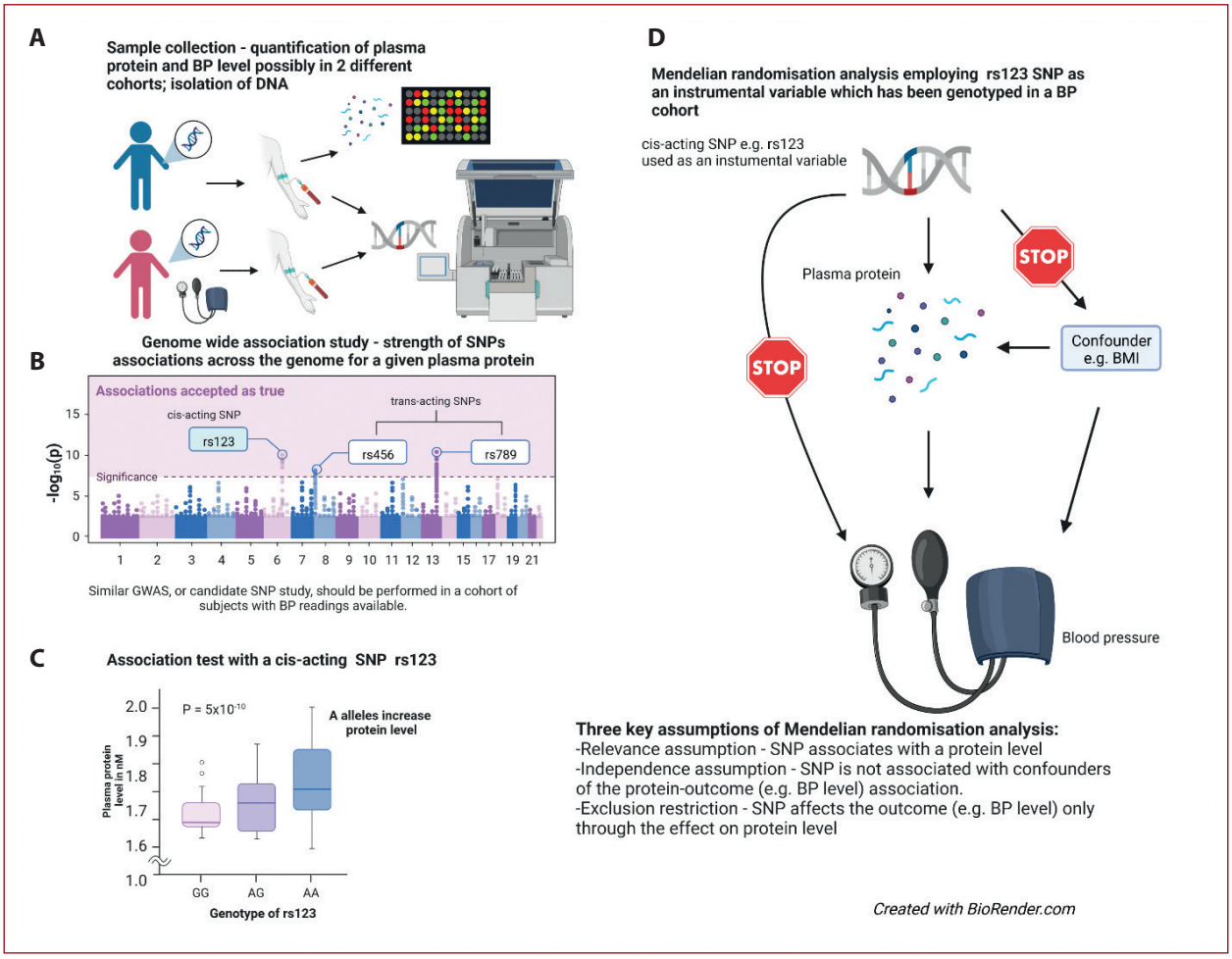

Figure 1. General design of a Mendelian randomization study aiming to assess the potentially causal link between a protein and disease outcomes. In two sample MR studies, 2 independent cohorts are genotyped and tested with respect to either exposure (e.g. biomarker level) or the outcome (e.g. level of BP) (**A**). Genome-wide association study is conducted to find single nucleotide polymorphisms (SNPs) associated with exposure (**B**). GWASs on the plasma protein level often identify both cis- (proximal to the gene coding protein of interest) and trans-acting genetic variants. The latter variants may be considered pleiotropic, thus the effect of cis-acting SNPs (**C**) is often used in the formal MR analysis (**D**). A similar approach may be applied to various other biomarkers such as lipid traits, amino acids, and other metabolites

Abbreviations: BMI, body mass index; BP, blood pressure; GWASs, genome-wide association studies; MR, Mendelian randomization, SNPs, single nucleotide polymorphisms

with CAD, but lipoprotein(a) appears to be an independent risk factor [22, 23].

BIOMARKERS OF HT AND ASSOCIATED CVDs — OLD AND NEW CANDIDATES

While all the above-described approaches provide evidence of association or even casual relation between studied biomarkers and disease outcomes, it is still hard to explore the exact time course of the observed molecular changes, whether they precede the onset, determine the early development, or are crucial for progression of HT and further predispose to other CVDs or determine all disease stages. Additionally, an altered level of a particular biomarker may drive progression of the disease, however, it may also serve as a compensatory mechanism, thus the studies showing associations should always consider both harmful or protective properties of the biomarker. Moreover, biomarker-biomarker or biomarker-environment interactions may also occur, and yet are difficult to study. Another considered vulnerability may be a regional heterogeneity of the biomarker level across the tissues and even within the same tissue. All these important aspects may affect further drug design process and impact the effectiveness of the proposed new treatment, thus careful and thoughtful planning of animal and observational studies is of great importance.

THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

Nevertheless, numerous relevant biomarkers have been related to hypertensive condition. The renin-angiotensin-aldosterone system (RAAS) plays a fundamental role in BP regulation, and RAAS inhibition is a basic strategy for HT treatment. While numerous polymorphisms in RAAS-related genes were tested for BP traits in the "candidate gene" research era, surprisingly first GWASs were not consistent

and rarely identified SNPs within the RAAS as relevant for BP variability [24]. Eventually, large-scale GWASs revealed a direct association between some of the SNPs in the genes encoding RAAS and BP traits, e.g. rs699 in the *angiotensinogen* (*AGT*)[25–27]. This underlies the necessity for performing large-scale studies to achieve adequate statistical power for detecting association, particularly for low-prevalent SNPs. However, it should be also pointed out that the association of RAAS genes with BP traits may be population specific [28].

The potential of RAAS proteins as biomarkers predicting new-onset of HT was long limited and not consistent between studies mainly due to the analytical challenges in protein measurement methods [29]. However, a recent study revealed that aldosterone-to-renin ratio obtained by simultaneous measurement of both analytes using fully automated chemiluminescence immunoassay (CLIA), possesses a relatively stronger predictive potential for incidental HT, compared to individual aldosterone or renin levels [30]. This novel analytical method may be used in the future in large-scale biobanks allowing testing causal relationships between RAAS proteins and BP traits.

Additionally, it is now well-established that the occurrence and maintenance of HT are accompanied by the strong activation of the brain RAAS, and pharmacological or genetic interruption of this system alleviates HT in animals [31]. The brain RAAS consists of all classical components (i.e. renin, aldosterone, angiotensinogen, peptidases, angiotensins, and their specific receptors); however, the major role is played by angiotensin III (Ang III), a product of angiotensin II (Ang II) cleavage catalyzed by a membrane-bound zinc metalloprotease — aminopeptidase A (APA). Ang III, similarly to Ang II, increases BP through sympathetic activation and stimulation of arginine-vasopressin release. Growing preclinical evidence of the protective effect of APA inhibition in HT led to the development of a first-in-class prodrug, RB150 or Firibastat, which crosses the blood-brain barrier following oral administration and is cleaved into EC33, an active APA inhibitor. The safety and efficacy of Firibastat in HT patients were confirmed in phase I, IIa, and IIb clinical trials, and the drug is now tested in phase III trials in treatment-resistant HT patients (NCT04857840, NCT04277884) [32, 33].

Genetic variation explains only a part of the trait variability. The epigenetic changes, defined as reversible modifications in DNA structure (and not sequence) controlling gene expression level, may represent a "missing heritability" in HT and associated CVDs. Indeed, hypomethylation of CpG in the *angiotensin I converting enzyme* (*ACE*) promoter and *angiotensin II receptor type 1* (*AGTR1*) genes was associated with higher SBP levels or HT in humans, respectively [34]. The epigenome-wide association studies are still developing, and further technological advance is needed to explore other than DNA methylation types of epigenetic mechanisms controlling gene expression level (e.g. histone modification or non-coding RNAs). Moreover, as epigenetic changes are determined by both genetic and environmental drivers, they cannot be used as IVs in MR analysis, hence excluding the possibility of testing their direct causal relationship with BP-related traits using this methodology. However, combining the results of GWASs with studies on methylation quantitative trait loci (mQTL) creates an opportunity to casually link DNA methylation in specific genes with BP and CVDs using MR approach [35].

THE NATRIURETIC PEPTIDE RECEPTOR 1 PATHWAY

Genetic research has contributed to the discovery of another important pathway significantly affecting BP levels in humans, confirming earlier studies in animal experimental models [36, 37]. In 2016, Liu et al. reported a novel, rare variant of *natriuretic peptide receptor 1* (*NPR1*) *rs35479618* associated with increased BP in an exome analysis. *NPR1* serves as a specific receptor for natriuretic peptides A and B (NPPA/NPPB) produced either by cardiac atria or ventricles, which lower BP by decreasing systemic vascular resistance and inducing renal sodium and water excretion. Subsequent large-scale GWASs confirmed the association of *NPR1* polymorphisms with BP traits as well as with CAD and additionally identified novel BP loci in *NPPA/B* genes [25–27, 38, 39]. Interestingly, a high circulating level of NT-proBNP (an inactive N-terminal proBNP) has been causally related to lower systolic (SBP) and diastolic BP (DBP) levels using MR analysis [40], which is in opposition to an earlier observational prospective study suggesting an elevated level of circulating NT-proBNP as a predictor of HT risk in normotensive subjects [41]. Additionally, higher NT-proBNP level has been independently associated with coronary heart disease (CHD), heart failure (HF), ischemic stroke, and cardiovascular and all-cause mortality across SBP, DBP, and pulse pressure (PP)-defined patient categories, and may serve as a biomarker of high CVD risk in patients who may benefit from intensive BP lowering strategies [42]. This example shows that study design may impact the conclusions drawn and underlines the necessity to combine all available evidence to define a role of a biomarker in a given disease. Importantly, there are ongoing clinical trials to assess the safety, tolerability, and pharmacokinetics of *NPR1* agonist drugs (REGN5381 [NCT04506645], REG9035 [NCT05291546]), which, if successful, will be further tested for HT treatment.

THE NITRIC OXIDE PATHWAY

The balance between vasodilation and vasoconstriction is crucial for the maintenance of the optimal BP level, and endothelial cells (ECs) are an important source of both types of vasoactive factors. Endothelial dysfunction (ED) has been recognized as a classical hallmark of HT in animal models and humans, preceding the onset and accompanying progression of the disease. Molecular mechanisms of ED are relatively well-studied. Most of all, ED involves decreased activity of the endothelial nitric oxide synthase

(eNOS encoded by *NOS3*) and thus reduced bioavailability of vasodilatory nitric oxide (NO). Importantly, large-scale GWASs identified polymorphisms in the *NOS3* gene as significantly correlated with BP traits [27, 38]. Although well explored, the therapeutic potential of targeting the NO pathway is unexploited, as no drugs targeting eNOS are currently in clinical use for systemic HT (reviewed elsewhere [43]).

THE ENDOTHELIN-1 PATHWAY

Oxidative stress, low-grade vascular inflammation, and hypoxia reduce NO bioavailability, inducing ED in CVDs [44] and evoking enhanced production of ET-1, encoded by the *EDN1* gene, by ECs, thus creating a vicious cycle of prohypertensive factors [45]. In dysfunctional ECs, ET-1 acts mainly as a potent vasoconstrictor, activating ET_A and ET_{B2} receptors on vascular smooth muscle cells. However, in lower concentrations, ET-1 possesses also counter-regulatory properties. ET-1 can induce NO production and prostacyclin synthesis by acting through the ET_{B1} receptor expressed on ECs. Meta-analysis of human studies investigating plasma ET-1 levels confirmed that HT patients are characterized by significantly higher ET-1 level compared to normotensive subjects [46]. Moreover, a higher level of plasma ET-1 may predispose normotensive subjects to develop HT [47]. Interestingly, combining the results from epigenetic, phenome-wide, and GWASs allowed to identify the intronic variant of *phosphatase and actin regulatory protein 1* (*PHACTR1*), associated with HT, CAD, migraine headache, cervical artery dissection, and fibro-muscular dysplasia, as a distal regulator of *EDN1* gene, uncovering the new control mechanism of ET-1 expression [48]. Additionally, an MR study revealed a potentially causal relationship between increased levels of plasma C-terminal-pro-endothelin-1 (CT-proET-1), a biomarker of ET-1, and an increased risk of ischemic heart disease (IHD) [49]. Although inhibition of the ET-1 pathway has been proposed as a BP-lowering therapy over thirty years ago, the utility of the first proposed drug candidates for systemic HT treatment has long been questionable due to adverse side effects. Eventually, the results of the PRECISION phase III trials (NCT03541174) published by Schlaich et al. [50] demonstrated both short-term and long-term (48 weeks) safety and efficacy of the aprocitentan i.e. a novel, oral, dual ET-1 receptor antagonist, added to the standardized antihypertensive treatment for resistant HT.

THE SPHINGOSINE-1-PHOSPHATE PATHWAY

Sphingosine-1-phosphate (S1P), a bioactive sphingolipid, has been recently recognized as a crucial regulator of BP homeostasis. Similarly to ET-1, it supports or opposes vasodilation through NO, depending on its concentration and tissue compartment [51–54]. Vasodilatory action of S1P is mediated by S1P type 1 and 3 receptors (S1PRs) expressed on ECs, and consequent eNOS activation. However, a high concentration of circulating S1P (as observed in HT in both animals and humans [55]) induces S1PR1 internalization, and facilitates binding of S1P to S1PR2/3 receptors, inducing vascular contraction. FTY720 (or fingolimod), a clinically approved drug for the treatment of multiple sclerosis, acting mainly by functional antagonism of S1PR1 and causing peripheral lymphopenia, primarily triggers transient BP decrease while chronic administration of the drug might result in the onset of HT [56]. Additionally, GWASs found SNP in the 3' UTR region of *S1PR2* as associated with BP indices, however, this locus contains other genes, such as *MRPL4* and *DNMT1,* thus identification of a true causal gene is challenging [27, 38]. The development of S1PRs-targeting drugs, and especially agonists of S1PR1 which do not mediate receptor internalization, seems to have an unexploited therapeutic potential in HT.

THE IMMUNE SYSTEM

While the contribution of the immune mechanisms to the HT pathophysiology is evident and many classical proinflammatory factors are increased in HT [57–60], immune-targeting therapies are still far from clinical use for systemic HT. Nevertheless, there are hypotheses that certain adverse effects could be controlled with proper design of the therapeutic strategy, so they would not exceed these for standard anti-HT treatment [57]. Recently, based on observational data and subsequent MR analysis, a potentially causal relationship between increased blood lymphocyte count and higher SBP and DBP in humans has been suggested. Furthermore, a reverse effect of higher BP on increased monocyte, neutrophil, and eosinophil counts has also been observed [61]. Moreover, MR analysis performed by Astle et al. [62] showed a positive potentially casual association between blood lymphocyte count and CHD, with no effect on chronic kidney disease (CKD). Importantly, this study has also provided additional evidence of the potentially causal role of the S1P pathway in CVD pathophysiology, as SNPs in *S1PR1* and *Sphingosine kinase 1* (*SPHK1*) has been associated with the absolute count of blood lymphocytes. Therefore, the above observations may serve as an interesting starting point to further investigate whether genetic variations in S1P-related genes are associated with tissue infiltration by immune cells and, if so, what are clinical consequences of such a phenomenon.

OTHER BIOMARKERS IDENTIFIED BY THE MR APPROACH

Genetic causal inference tests have been used to verify the direction of association of many other biomarkers for HT and associated CVDs. For example, MR was used to demonstrate the causal negative effect of *SH2B adaptor protein 3* (*SH2B3*), one of the top GWAS-identified loci for the level of BP and CAD, on circulating β-2-microglobulin level, which was associated with prevalent and incidental HT [63]. MR analyses found that higher levels of sex hormone binding globulin (SHBG), testosterone, iron, and insulin-like growth factor 1 (IGF-1) appeared protective

for the development of coronary atherosclerotic outcomes and HT [64–66], while high plasma levels of uric acid, uromodulin (UMOD), and alanine aminotransferase (ALT) have been potentially causally related to adverse cardiovascular outcomes and HT [65, 67, 68]. On the other hand, MR did not support the presence of a causal link between plasma homocysteine level and BP [69], galectin-3 (GAL-3) levels, and CAD-related mortality [70], or CRP (C-reactive protein) and vitamin D level and CAD and its risk factors [65, 71, 72]. However, supplementation with vitamin D seems to offer promising support for cardiovascular health [73]. Moreover, MR suggested potentially causal effects of plasma factor VIII (FVIII) activity levels on venous thrombosis and CAD risk, and plasma von Willebrand factor (VWF) levels on ischemic stroke risk [73], while cardiac troponin I (cTnI) and cardiac troponin T (cTnT) appear to be risk factors for atrial fibrillation, but not for CAD, stroke, or HF [74]. On the other hand, a growing body of genetic-based evidence suggests a potentially causal role of matrix metalloproteinases (MMPs), and especially MMP12, in the occurrence of stroke [18, 75, 76].

High-throughput profiling of plasma proteins has additionally found that levels of adrenomedullin (ADM), urokinase-type plasminogen activator (uPA), interleukin 16 (IL-16), cellular fibronectin (cFN), and insulin-like growth factor-binding protein 3 (IGFBP-3) were associated with BP level [40], while levels of stromal cell-derived factor 1 (CXCL12) and macrophage colony-stimulating factor 1 (CSF1) were associated with CAD, next to the established risk factors, i.e. lipoprotein(a), apolipoprotein E, and interleukin 6 receptor [77].

While polygenic risk scores constructed using GWASs identify individuals at risk of developing disease in their lifetime, causal biomarkers may additionally inform on disease progression and treatment efficacy. Therefore, in the future, the above-described biomarkers, identified as potentially causally related to CVDs in MR studies, may become useful for diagnostic evaluation, treatment monitoring, or risk stratification. However, when possible, they should be further evaluated in prospective cohorts or clinical trials. The pharmacological targeting of the identified biomarkers may also be of therapeutic importance, however, possible pleiotropy due to the effect of a genetic variant on other proteins, than the ones that have been identified, has to be assessed. Importantly, availability of the large-scale, phenome-wide studies that collect information on thousands of clinically relevant traits, will also facilitate identification of side effects related to targeting the protein of interest, including potentially beneficial or harmful effects on diseases other than initially investigated.

HT AS A MODIFIABLE RISK FACTOR FOR OTHER CVDs — EVIDENCE FROM THE MR STUDIES

The importance of BP lowering therapy is evident as chronically increased BP is a major risk factor for many life-threatening CVDs. Importantly, Higgins et al. [78] in their recent study using UK Biobank data showed that a 5, 10, and 23 mmHg decrease in SBP in a population significantly decreased associated morbidity by 17%, 31%, and 56%, respectively. Moreover, MR analysis provided evidence that high SBP was a causal risk factor for aortic valve stenosis, ischemic stroke, dilated cardiomyopathy, CAD, subarachnoid hemorrhage, ischemic cerebrovascular disease, endocarditis, hemorrhagic stroke (all types), chronic kidney disease, HF, transient ischemic attack, atrial fibrillation (AF), rheumatic heart disease, and peripheral vascular disease, as well as intracerebral hemorrhage (ICH) and aortic aneurysm (listed in order from the highest casual estimate value), while an inverse causal estimate was demonstrated for deep vein thrombosis [78]. Some MR studies aimed to distinguish between SBP and DBP-driven effects on CVD risk. While both SBP and DBP were individually causal for various CVDs, simultaneous analysis of both BP indices using a multivariable MR approach found that SBP was a major risk factor for CAD, stroke, HF, and atrial fibrillation, while the association of DBP with CVDs became null after adjustment for SBP [79]. Similar results were noted by Surendran and colleagues who additionally found a potentially protective effect of DBP on large artery stroke when adjusting for the effect of SBP [80]. While the most commonly used univariable MR aims to test the total effect of single exposure on the outcome, multivariable MR is currently considered an excellent tool to dissect the direct effects of multiple correlated traits such as lipid classes, BP indices, or obesity parameters on various complex diseases. This makes multivariable MR a relevant analytical method for analysis of biomarkers that often correlate with each other.

Besides pharmacological targeting of high BP, lifestyle modification may significantly help to keep BP on the normotensive level. For example, the MR approach demonstrated that HT may be a consequence of increased triglycerides, body mass index, alcohol dependence, smoking initiation, and insomnia while the increased level of high-density lipoprotein cholesterol (HDL-C) and higher educational level of patients were significantly associated with lower odds of HT [81]. Notably, combining knowledge of genetic proxies for drug targets and MR analysis may be used to provide information on the efficacy and side effects of drugs including antihypertensive medications, statins, and proprotein convertase subtilisin/kexin 9 (PCSK9) inhibitors. This has led to conclusions on the null effect of antihypertensive medications on Alzheimer's disease [82], potentially harmful effects of calcium channel blockers on diverticulosis risk [83], and ACE inhibition on colorectal cancer risk [84]. Notably, genetically proxied effects of statins and PCSK9 inhibitor use have been associated with increased risk for type 2 diabetes [85, 86], and statins potentially increased risk for ICH [87], which is consistent with a genetically defined potentially causal link between elevated LDL-C and lower risk of ICH [88]. Additionally,

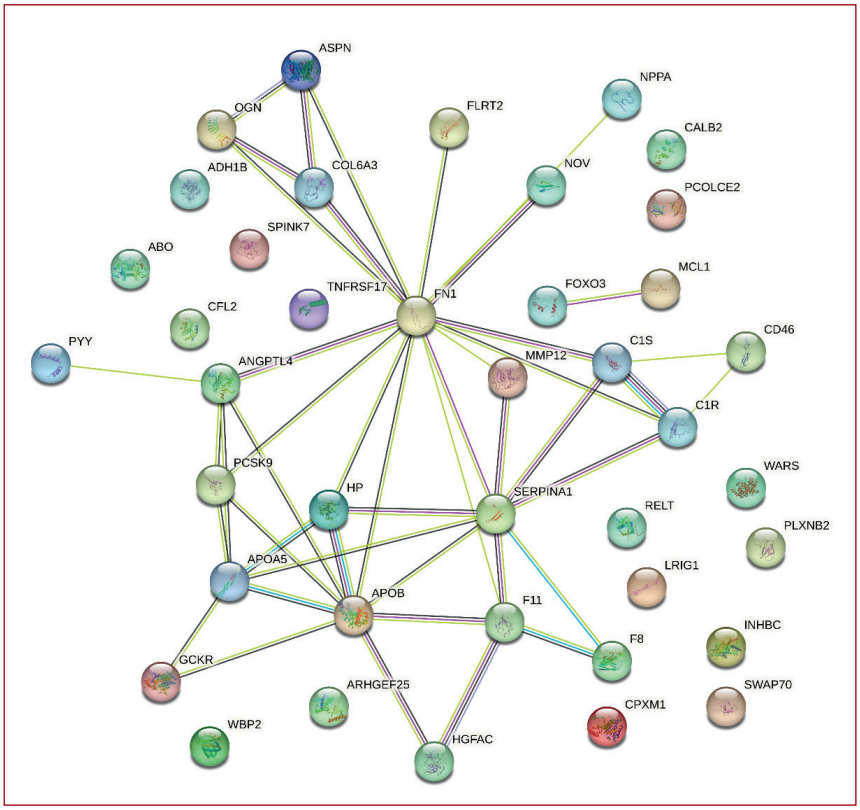

Figure 2. Genes sharing plasma cis-protein quantitative trait locus (cis-pQTL) with causal variant identified by genome-wide association studies (GWASs) investigating BP indices, CAD, atrial fibrillation, coronary artery calcification, and stroke. Between-protein connection based on various levels of evidence was performed by STRING software [103–104]. Information on plasma cis-pQTLs and related GWAS traits was derived from the DECODE consortium [90]. Presented genes encode proteins that are established risk factors for CVDs (e.g. PCSK9, APOB) or plausible candidates for further causal tests using, e.g., MR framework to establish a relationship between certain proteins and CVDs

Abbreviations: CAD, coronary artery disease; CVD, cardiovascular disease; other — see Figure 1

statins, but not PCSK9 inhibitors, were potentially causal for adverse neurocognitive outcomes [89]. While the benefit of statin use is well documented in various CVDs, these findings further suggest that the choice of particular lipid-lowering therapy may become more personalized in the near future.

EMERGING PROTEOMIC AND METABOLOMIC TOOLS FOR TESTING CAUSAL INFERENCE IN RELATION TO BP AND RELATED CVDS

High-throughput and well-powered metabolomic and proteomic analyses have been recently conducted in DECODE [90], Fenland [91], UK Biobank [92], and other studies utilizing technologies such as Nuclear Magnetic Resonance (NMR), antibody-based OLINK, or aptamer-based SomaScan platforms. Plasma NMR-based metabolomics helped to identify amino acids potentially causally associated with BP and CAD. Genetically proxied plasma glycine level appears to be protective against the development of CAD and HT, while branched chain amino acids (i.e. valine, leucine, and isoleucine) are genetically associated with elevated BP levels [93–96]. Importantly, analyses restricted to genetic variations in genes directly involved in glycine metabolism found no causal effect on the level of BP, which suggests possible pleiotropic effects (i.e. acting through other than glycine factors) of remaining genetic variations on BP level.

OLINK and SomaScan proteomic technologies vary in terms of specificity and precision [97] and possess certain limitations such as significant influence of protein-altering variants (e.g. missense or splice variants) on the antibody/aptamer binding. Thus genetic variations associated with the level of a certain protein (i.e. pQTLs-protein quantitative trait loci) are often cross-validated with mRNA expression QTLs (eQTLs) derived from, for example, GTEx [98] or eQTLgen [99] consortia. Nevertheless, proteomic studies using novel high-throughput technologies allow for simultaneous quantification of thousands of plasma proteins in a single sample and consequently deliver a huge amount of information on the human genome-proteome-phenome relationship. Published studies often report results of colocalization of genetic signals related to both protein level and particular trait/disease of interest, e.g. BP or CVDs (Figure 2), or even results from formal MR analysis used to infer causal direction from observed colocalization. Such analysis has been performed in the SCALLOP Consortium, and it demonstrated that genetically proxied levels of IL6RA and placental growth factor are potentially causal for the development of CAD [18].

Although "omic" and MR studies generate a plethora of data that greatly expand our knowledge on cardiovascular pathophysiology, their results have to be always considered in the context of the studied population and genetic pleiotropy (Figure 1) that may bias MR studies, and they should not be extrapolated to other populations without prior verification [100]. The overrepresentation of Europeans in genetic studies is a recognized issue of significant concern since it has implications for disease management in the global population [101]. This underlines the importance of the collaboration between scientists, with the NHLBI

TOPMed Program [102] or the SCALLOP Consortium as examples of the efforts to overcome the issue of missing ethnic/geographical diversity in "omic" studies.

CONCLUSIONS

The progress in research on the molecular background of HT and associated CVDs made in recent years is mostly a consequence of the rapid advancement in genomic, proteomic, and metabolomic studies. Well-designed and large-scale "omic" studies may serve as input data for studies employing MR approach, which enables testing potentially causal relationships between various molecular factors and disease outcomes. MR analysis may serve as additional evidence for observational studies and thus may improve the process of selection of drug candidates for clinical testing by excluding non-causal markers. Novel technologies and analytic tools accelerate the identification of novel molecular biomarkers which may soon become drug targets and ultimately help to slow down the incremental global burden of HT and related CVDs. Importantly, these technologies facilitate identification of CVDs that can be potentially targeted by already known drugs, not initially designed to treat particular CVD. Owing in part to the newest technologies, promising therapeutic strategies are under clinical investigation (e.g. brain RAAS inhibitors, NPR1 agonists, ET-1 receptor antagonists), which may prove beneficial in difficult-to-treat HT cases and may contribute to a decline in CVD-related mortality. However, the biomarkers most recently identified by the MR approach are awaiting clinical verification.

Article information

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