

Omics studies in hypertension and heart failure: pathways to precision medicine

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Abstract

Hypertension and heart failure are complex cardiovascular disorders. High blood pressure is one of the most important risk factors for heart failure but both conditions also share key pathogenetic mechanisms. For example, dietary salt intake, renal sodium excretion and storage of sodium in cardiovascular tissues plays a role in the development of hypertension and may also contribute to the development of heart failure, in particular to heart failure with preserved ejection fraction. Interindividual differences in the disease processes and heterogeneity in contribution of pathogenetic factors to the development of hypertension and heart failure pose challenges to researchers and clinicians alike. Omics technologies enable us to screen for large numbers of genetic variants, transcribed genes, and expressed proteins and metabolites. In this article we will share our views on how different omics technologies can provide information on the origins of hypertension and heart failure and why despite common pathogenetic principles we may expect differences in the performance of genomic and proteomic studies in these conditions. Where the right technologies are chosen, omics studies have the potential not only to unravel the pathogenesis but also to guide diagnosis and tailor treatment of patients with hypertension, heart failure and other cardiovascular diseases.

Keywords: hypertension, heart failure, omics, proteomics, genomics, salt, sodium

Introduction

Hypertension remains a major global health challenge, particularly in low and middle income countries where the prevalence is increasing. Even in high income countries where there has been some progress over the last

two decades, more than two thirds of patients with hypertension have suboptimally controlled blood pressure [1]. Hypertension is both directly and indirectly associated with cardiovascular diseases including chronic kidney disease, coronary artery disease and stroke, and is an important cause of heart failure. Notably, older adults account for the majority of hypertension-related morbidity and mortality—largely due to higher prevalence among the elderly [2,3].

In recent years we have witnessed enormous technological developments in genotyping, which have been paralleled by advances in measurement of large numbers of proteins and metabolites in biological sam-

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ples. In contrast to hypothesis driven research that focusses on a limited number of genes, molecules and pathways the progress in omics technologies has enabled researchers to conduct large scale screens of the genome, proteome and metabolome where factors associated with specific clinical conditions can be discovered without an a priori hypothesis [4,5]. In this article we will explore the challenges of omics research in hypertension and heart failure and the potential of these exciting new technologies to further our understanding of disease pathophysiology, thereby leading to development of novel treatments.

Clinical aspects of hypertension and heart failure

Our ability to characterise the molecular make-up of certain diseases has improved enormously, reaching a level where precise large-scale genomic and molecular information can be obtained quickly and at relatively low cost. In contrast, the clinical characterisation of cardiovascular diseases such as hypertension and heart failure remains relatively imprecise. It is important to understand the discrepancy between molecular and clinical phenotype in order to correctly interpret the results of omics studies in cardiovascular diseases.

Hypertension is somewhat arbitrarily defined but the majority of guidelines agree on a diagnostic threshold of 140/90 mmHg. However, further definitions such as "high normal blood pressure", different stages of hypertension severity, and varying definitions of hypertension in people with comorbidities such as diabetes or renal disease between guidelines indicate that "hypertension" is not a simple dichotomous phenotype. Blood pressure can be studied quantitatively or as a qualitative trait. However, sphygmomanometric brachial blood pressure measurement, which has been employed for the majority of clinical trials and omics studies, is not a precise reflection of "true" blood pressure, does not assess the pathophysiologically more relevant central blood pressure and only provides a snapshot of blood pressure as opposed to 24-hour ambulatory monitoring and analysis of blood pressure variability.

The European Society of Cardiology defines heart failure as "a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fa-

tigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral oedema) caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress" [5]. According to this definition there can be a wide range of clinical phenotypes in patients with heart failure, pointing again to discrepancy between the precise molecular characterisation and less distinct clinical phenotypes.

In contrast to hypertension, data on hospitalised patients suggest a decline in the incidence of heart failure over the last decade [6]. However, mortality rates remain high, reflecting a progressive increase in the proportion of cases with preserved ejection fraction (HFpEF) at the expense of heart failure with reduced ejection fraction (HFrEF) at a rate of around 1% per year [7-10]. Despite an overall slightly worse prognosis, patients with HFrEF can at least derive benefit from effective treatments positively impacting on survival over the last 30 years; in the case of HFpEF no current treatments have been shown to impact on mortality, which is often driven by non-cardiovascular conditions [5,11]. This is due to a complex and heterogeneous pathophysiology, primarily driven by comorbidities. Hypertension is the most prevalent of these comorbid conditions (76-96% of cases) and, through development of hypertensive heart disease, is a precursor to overt HFpEF [12]. Since patients with HFpEF are older than those with HFrEF, consistent with the demographic trends also observed in hypertension, the progressive aging of western societies renders HFpEF a prominent public-health problem and a research priority.

Pathogenesis

By nature of their unbiased approach, omics studies have the potential to discover novel pathogenetic principles. In this context it is important to briefly review what is already known about the mechanisms underpinning hypertension and heart failure.

Clearly, readers of this journal do not need to be taught about the pathogenesis of hypertension. It is important to bear in mind that blood pressure is generally determined by cardiac output (including intravascular volume, cardiac and renal function) and vascular tone and structure, however the contribution of these com-

ponents varies between individual patients with hypertension. In the extreme forms of secondary hypertension, single pathogenetic factors can be responsible for a patient's high blood pressure, whereas in patients with essential hypertension a variety of factors contributes to hypertension at various degrees [4]. Ultimately, factors such as the renin-angiotensin-aldosterone system, the sympathetic nervous system, salt intake or inflammation all act on the basic principles of volume/flow and vascular tone.

Similarly, heart failure can result from a wide range of clinical conditions that affect the heart. Whilst a majority of patients develop heart failure secondary to myocardial infarction, other myocardial disease as well as abnormalities of the valves, pericardium, endocardium, heart rhythm and conduction can also cause heart failure [5]. Each of these specific factors can have multiple aetiologies, and in individual patients combinations of these factors together determine the clinical phenotype. Therefore, heart failure is also far from being a uniform clinical condition, and such variability in the clinical phenotype is clearly a challenge for molecular studies into precise mechanisms.

Salt: a pathogenetic principle in both hypertension and heart failure

A factor that is shared between the pathogenesis of hypertension and heart failure is salt. We provide a brief overview of the role of salt to highlight two main aspects. First, both blood pressure and cardiac function can be modified by external (environmental) factors including salt intake but also diet in general, exercise and smoking. Second, not all people will be affected by external factors in the same way.

The close association between salt and blood pressure has been known for many decades. On a population basis, this appears to be most pronounced with increased age, in persons consuming high-sodium diets (i.e. > 5 g Na⁺/day) and in those with hypertension [13]; interventional studies decreasing salt intake proved effective in lowering blood pressure [14], although the optimal range of sodium intake for cardiovascular health is still under debate [15]. In individuals, "salt-sensitivity of blood pressure" reflects marked heterogeneity of BP response to salt loading [16]; whilst substantial salt sensitivity of blood pressure is restricted

to only a minority of the population, the majority only experience mild blood pressure variation with changes in sodium intake. Despite arbitrary cut-offs for this response [17] the definition of salt sensitivity allowed the identification of different factors involved in sodium balance, including the renin-angiotensin-aldosterone system, endothelins, oxidative stress, sympathetic nervous system and natriuretic peptides, among many others [17]. Such heterogeneity offers an opportunity to identify the individual genetic and molecular regulators of response to salt and thereby the best possible treatment in the sense of precision medicine. Beyond the impact on BP values, increasing evidence suggests that salt intake may also have a direct effect on other important mediators of heart failure, such as left ventricular hypertrophy and diastolic dysfunction, both directly and independently of its pressor effect [18,19].

Novel concepts suggesting cycling variations of sodium excretion compared to intake [20] and local accumulation in tissues [21-23] led to the identification of a close association between hypertonic skin sodium content and left ventricular hypertrophy [24]. Whilst generally thought to be a secondary phenomenon due to inadequate haemodynamics in heart failure and activation of compensatory neurohormonal mechanisms [25], primary abnormalities in local or systemic sodium metabolism could also play a key role in progression to heart failure.

It is important to note that hypertension and heart failure share a number of pathogenetic principles of which altered sodium handling and storage is only one. Unbiased approaches to discover such principles can lead to better understanding of complex cardiovascular diseases. Omics technologies offer such opportunities.

Omics

The genetic code within DNA is transcribed into RNA and translated into proteins that serve as building blocks, signalling molecules and enzymes which regulate metabolism. With less advanced techniques researchers in the past were only able to assess a small number of genetic variants, proteins and metabolites. In contrast, modern technology facilitates the detection of millions of genetic variants or even characterisation of the whole genome [26,27]; similar techniques exist for characterisation of mRNAs and thereby the tran-

scriptome. On a protein and metabolite level, mass spectrometry and nuclear magnetic resonance spectrometry, the miniaturisation of antibody-based detection methods and development of antibody-free methods enable us to characterise a large number of proteins and metabolites in tissue samples and body fluids. Moreover, closer insights into regulatory processes including modulation of transcription and translation by non-coding RNAs, epigenetic factors such as DNA methylation and posttranslational modification of proteins have been dissected in detail and can be assessed on a large scale [28-30].

Cardiovascular diseases develop as a result of the interaction between genetic and environmental factors. In rare diseases such as monogenic forms of hypertension or dilated cardiomyopathies the influence of genetic factors can be particularly strong with environmental factors playing a smaller role, whereas in common diseases such as essential hypertension and coronary artery disease the interaction between multiple genes with small individual effects and numerous environmental factors is more complex. It is therefore evident that complex cardiovascular diseases cannot be precisely described by genetic factors whereas the transcriptome, proteome and metabolome as well as epigenetic features can better reflect interactions with the environment and could allow a precise description of disease state.

Genetic features are in the first instance risk factors or factors that outline the potential of an organism to develop disease, but their value for predicting disease in individual people depends on the complexity of the disease's genetic make-up, environmental influences and the heterogeneity of the phenotype. We will briefly outline the role of genomics and "higher omics" such as proteomics and metabolomics in hypertension and heart failure.

Genetics and genomics in hypertension and heart failure

There are a number of common challenges in genetic studies of hypertension and heart failure. First, both are conditions affecting the elderly and if a study participant is apparently disease free at the time of study they may still develop disease later in life, meaning their genetic make-up would have been wrongly associated

with "control" rather than "disease" status. Second, and related to the first factor, both hypertension and heart failure are characterised by many years of silent disease development before clinical symptoms occur. Precise phenotyping of early subclinical features rarely occurs in largescale genetic studies due to logistic and financial constraints. From these indirectly results a third point. Hypertension and heart failure are common conditions, and without precise characterisation and information from long-term follow-up "controls" may be mislabelled and in fact be "cases" if one would have more information on these study participants.

This "caseness of controls" has been identified as one of the main reasons why the original Wellcome Trust Case Control Consortium study into the genomics of hypertension has found no genetic variants that were significantly associated with hypertension; a substantial number of the non-phenotyped control subjects may have had hypertension and thus diluted the power of the study [31]. This challenge can be overcome by larger sample sizes where this dilution effect is compensated by greater numbers, by at least basic phenotyping of controls and by selection of extremes of phenotypes in order to inflate the odds ratios that can be observed at a given sample size. All of these strategies have been highly successful in contemporary genomic studies in hypertension [32-34] and led to the discovery of genetic features robustly associated with hypertension and blood pressure [35].

In contrast, there has been little success from genome wide association studies into heart failure. Whilst there are deep insights into the genetic make-up of animal models with specific and well characterised phenotypes [36] the situation in humans is more complex [37]. The likely reasons have already been discussed above: heart failure is the result of a combination of clinical conditions and environmental factors where each of the conditions (hypertension, coronary artery disease, heart rhythm etc.) is multifactorial with complex genetic determinants. Nevertheless a few genetic factors such as the gene encoding Hypocretin/Orexin Receptor-2 [38] or loci on chromosome 5q22 [39] and elsewhere [40] have been found to be associated with heart failure. Similarly, genetic studies have been successful in conditions where the phenotype is well defined (e.g. left ventricular dimensions) or when specific conditions such as dilated cardiomyopathies have been studied [37].

Higher omics in the diagnosis of hypertension and heart failure

The phenotype "hypertension" can be easily assessed by measurement of blood pressure. Early stages of hypertension such as "high normal blood pressure" or "pre-hypertension" that are associated with greater risk of progression to over hypertension and may be subject to preventative therapies [41] can also be assessed by measurement of blood pressure with different predetermined thresholds. It is not easy to imagine how complex omics-based tests can add to or even replace a clinical tool as simple as a sphygmomanometer. Still, a genetic risk score for hypertension combined with information from transcriptomics, proteomics and metabolomics to inform if and when the genetic risk evolves into early disease may be useful to target preventative and therapeutic strategies. Beyond this we can imagine a role of these technologies in the assessment of hypertension-related organ damage [4].

This is different in heart failure where the disease is defined by structural and functional changes of the heart that directly affect its protein composition and function and hence the metabolism of other organs. One would therefore expect that proteomic and metabolomic studies can reveal clinically important diagnostic information on cardiac structure and function in patients with heart failure. Indeed, we already use proteins such as natriuretic peptides and cardiac troponins for diagnosis of heart failure and related conditions. Natriuretic peptides are produced by cardiac tissue in response to volume overload and ventricular wall distension. Therefore, they are elevated in many clinically overt or preclinical heart diseases mediated by ischemia, hypoxia, and/or fibrosis. In people without baseline cardiovascular disease, these biomarkers were strongly predictive of mortality and cardiovascular events [42,43], particularly first-onset heart failure [44]. A similar predictive value in a high risk hypertensive population was paralleled by a reduction in cardiovascular risk specifically provided by those antihypertensive drugs that lowered N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels below a specified cut-off [45].

Elevated levels of natriuretic peptides have also been observed in preclinical diastolic dysfunction [46], and omics approaches could help to understand the link between this cardiac parameter and progression to

heart failure. By studying a large number of molecular features at the same time, omics techniques can indeed cover the whole spectrum from early to advanced disease stages. We will give a specific example from the area of urinary proteomics but will first briefly summarise the links between hypertension and HFpEF.

Hypertension, diastolic dysfunction and HFpEF

HFpEF has historically been referred to as "diastolic heart failure", i.e. heart failure due to ventricular diastolic dysfunction (impaired relaxation and increased diastolic stiffness) that can be present at rest or induced by stress. As mentioned above, hypertension is the most prevalent comorbidity in HFpEF and appears to actively contribute to its development. Nevertheless, research over the past decade identified the complex interplay of multiple other determinants including peripheral mechanisms such as microvascular inflammation with oxidative stress, endothelial dysfunction, vasculature stiffness and impaired vasodilation, rarefaction and impaired diffusion of oxygen in skeletal muscle [12]. Notably, hypertension as well as ageing are known to affect many if not all of these components. Through accumulation in local microenvironments and activation of multiple mediators of the immune system [47], which itself plays a key role in development of hypertension [48,49] and heart failure [50], sodium might contribute to both hypertension and HFpEF.

Urinary proteomics in diastolic dysfunction and heart failure

In people without kidney disease urine contains only small amounts of selected proteins such as uromodulin (Tamm-Horsfall protein) or albumin. There are, however, a large number of smaller polypeptide fragments that are freely filtered in the glomeruli or actively secreted in the renal tubules. These polypeptides can be accurately assessed by proteomic techniques such as capillary electrophoresis coupled to mass spectrometry [51].

Whilst individual urinary peptides (via the source protein from which they derive) can provide important information on specific molecular pathways, they can

also be combined to produce signatures of various clinical conditions. We and others have shown that diseases including chronic kidney disease [52], coronary artery disease [53] and bladder cancer [54] are associated with differences in the urinary peptidome compared to healthy individuals. Out of thousands of peptides in the urine these mass spectroscopic signatures include 200 to 300 differentially expressed peptides that can be visualised graphically and transformed into a numerical value.

A first study by Kuznetsova et al. [55] established the concept that a urinary peptidomic signature of diastolic dysfunction can be established and that this signature is also different in people with overt heart failure compared to control subjects. Subsequently we and others have shown that such signatures can predict cardiovascular and cardiac outcomes in a general population [56], can aid in the diagnosis of HFrEF [57], predict incident heart failure [58] and provide pathophysiological links between diastolic function and markers of collagen synthesis and degradation [59]. It is clearly too early to propose a clinical role for omics-based tests in routine practice but progress especially in the area of urinary proteomics is promising.

Omics as a tool for precision medicine in hypertension and heart failure

In our article we have focussed on omics techniques in the diagnosis of hypertension and heart failure and its prognosis and complications. These are, however, clinical scenarios where omics-based tests remain removed from clinical implementation – simply because they "compete" with less complicated, better standardised and less expensive existing tests such as measurement of blood pressure, measurement of natriuretic peptides and echocardiography.

Omics techniques may be much closer to clinical use in the area of precision medicine. In precision medicine the best treatment at the right cost will be offered to the right patient, enabling greatest therapeutic action with minimal adverse events [60]. We would assume that in the management of hypertension genomic approaches will be useful by defining individual risk and genetically driven response to treatments in the sense of pharmacogenomics. Transcriptomic, metabolomic and proteomic approaches together with

genomic information may in the future define an individual's molecular blood pressure phenotype and help to further refine preventative and therapeutic approaches. In heart failure there are ongoing projects including the EU funded programmes "HOMAGE" [61] and "BIOSTAT-CHF" [62] that employ a range of omics technologies to describe the molecular make-up of patients with heart failure in order to precisely direct existing therapies and to develop novel therapeutic strategies.

Summary and Conclusions

Complex diseases such as hypertension and heart failure can only be understood by studying their molecular make-up comprehensively. Despite all challenges, omics technologies offer the right tools to dissect the pathophysiology of these cardiovascular conditions, to help in clinical diagnosis and prediction of outcome, and to target therapeutic approaches. Other medical disciplines such as oncology are ahead of the game but in cardiovascular, metabolic and renal diseases the clinical and scientific communities have also realised the potential of omics techniques. An ongoing randomised clinical trial to prevent diabetic nephropathy stratifies patients to the treatment arm based on a urinary peptidomic signature [63] and we would expect that similar approaches can be employed in hypertension and heart failure.

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Conflict of interests

None.

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