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Systems biology to battle vascular disease

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Vascular disease (VD), whether manifested as atherosclerosis or arteriosclerosis in cerebrovascular, coronary or peripheral artery disease, represents the major burden of the health systems due to the high prevalence and is the main cause of mortality in both the male and female populations of the European Union. In addition, VD accounts for high costs both on a *per capita* basis and on a total basis, and financial consequences are estimated to be just under 110 billion per year (http://www.heartstats.org). The correlation of the different final stages of VD with genetic variants has been noted in several studies (e.g. [1,2]). VD with its micro- and macrovascular manifestations is especially prominent and especially accelerated in chronic kidney disease (CKD) [3] and diabetes mellitus [4]. Both CKD and diabetes are characterized by the highest morbidity and mortality rates due to VD of any cohort studied, and the role of VD has only recently been appreciated in early stages of CKD and diabetes. Unfortunately, current understanding of the pathophysiology of the micro- and macrovascular complications, especially of the early events responsible for disease onset and progression, is still limited mainly because of its enormous complexity and its polygenic and multifactorial traits. As a consequence, the therapeutic options are still limited. Detailed knowledge of the underlying biology and initial molecular pathophysiological events may enable early detection, definition of new and more appropriate therapeutic targets, and subsequently provide specific targeted therapies.

Patients with CKD are of special interest, as their pronounced and accelerated VD may serve as a model to elucidate its general pathogenesis. VD has been attributed to the increases of oxidative and glycaemic stress, microinflammation, endothelial dysfunction, sympathetic and renin–angiotensin overactivity [5–7]. Furthermore, CKD would present an interesting model to study VD as decreased renal function combines (i) accumulation of substances which physiologically undergo renal excretion, (ii) reduced synthesis of active substances and (iii) reduced renal metabolism of substances. Studies on the pathogenesis of VD in CKD have predominately focused on individual factors and mechanisms. However, it is most unlikely that single factors of mechanisms account for all there is to VD in CKD and in general. A huge amount of data on VD is available, but still mostly exist as specific observation in particular cohorts or as genomics, proteomics/peptidomics or metabonomics analyses on selected samples (e.g. [8,9]). These data are by no means complete yet, and need to be combined. For example, longitudinal and cross-sectional cohorts of patients with VD are available (e.g. [10,11]), but are currently only explored as single cohorts. However, the existing data on VD from human studies and animal and cellular models, and the additional targeted gathering of 'omics' data in clearly identified VD in conjunction to the recent advancements in systems biology analysis now provide the opportunity to model the onset and progression of vascular events in diabetes and CKD.

To characterize the causal chain of events and identify its mediators that may be useful as surrogate biomarkers, an integrative approach combining heterogeneous experimental data, existing prior knowledge and complex systems modelling presents itself as a valid route to deciphering path-

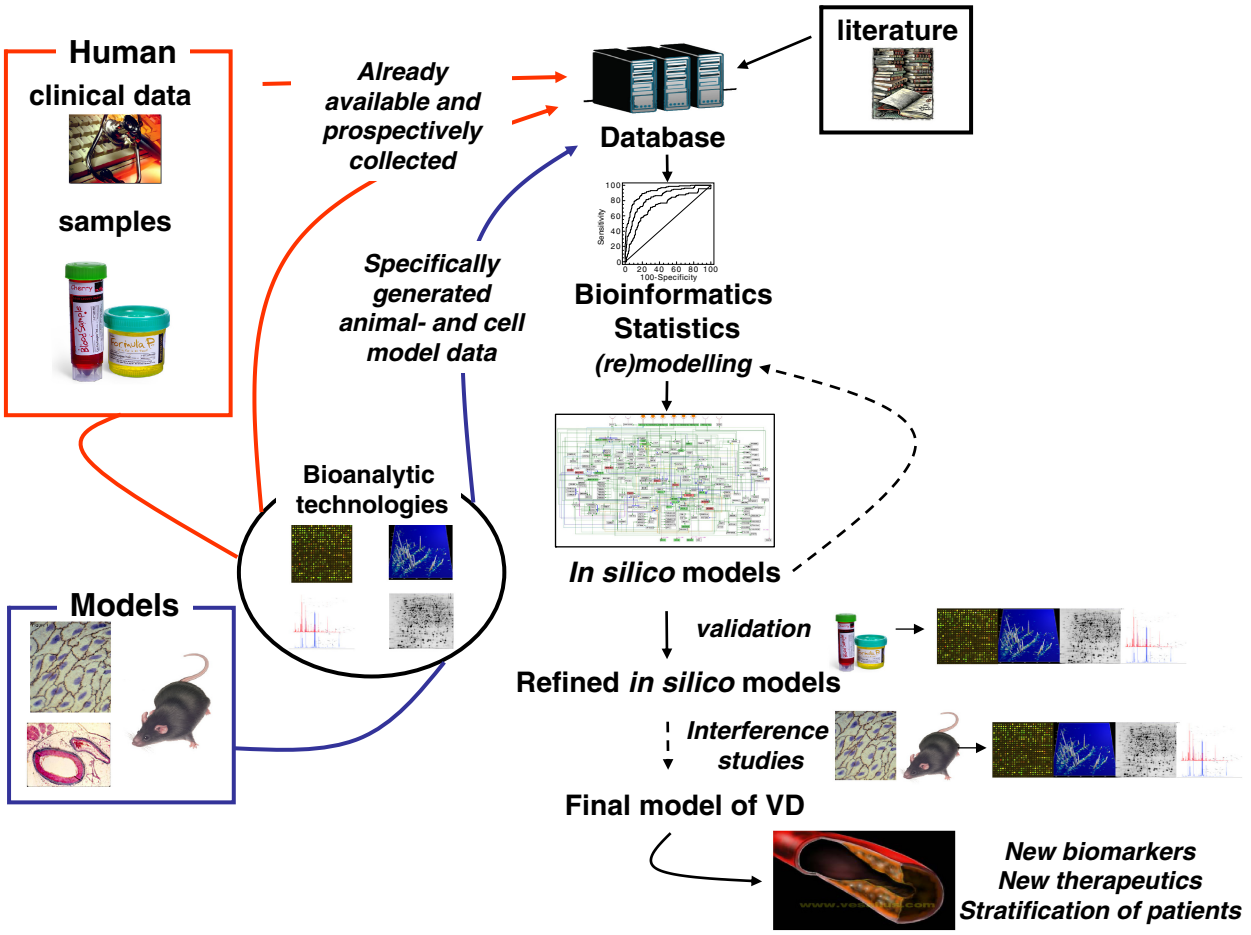

Fig. 1. Existing disparate data (clinical, –omics and literature) of human VD are being identified and combined, allowing the construction of a VD database. This will lead to the identification of missing data that subsequently can be prospectively collected. For example, it is anticipated that the existing datasets contain little data on the early VD events. In parallel, –omics data on animal and cellular models of VD will be collected to determine to which extent the processes are similar in humans and in these models. This in turn will allow us to progress with appropriate interference studies for validation of pharmacological interventions. All data will be analysed with appropriate bioinformatics and statistics, fed into the systems biology modelling process to produce the initial *in silico* model of VD. This initial model should be subsequently refined by the mandatory validation in human VD, followed by validation in animal and cellular models of VD disease. Multiple iterative steps will most be necessary to reach the appropriate final VD model generating new biomarkers, therapeutics and assessment of VD disease.

ophysiology of VD on a molecular level. A tight cross-talk between experiment and modelling is needed to allow identification of relevant molecular pathophysiological mechanisms, also with respect to timing of events and unearthing potential biomarkers and therapeutic targets in vascular lesions in diabetes and CKD. A possible way forward for data gathering and the use of systems biology to obtain useful real-life models for VD is graphically depicted in Figure 1. For details on systems biology approaches and the remaining hurdles, please consult the accompanying editorial on systems biology (doi: 10.1093/ndt/gfq033).

First reports using a systems biology approach to identify biomarker candidates for cardiovascular disease in CKD [12], and for the mapping of pathways in cardiovascular disease [13] have been published. These approaches enabled the identification of networks of proteins associated with vascular disease. These initial results also clearly highlight a major obstacle for systems biology: a significant lack of high quality and standardized -omics datasets, enabling inter-study comparison [13–15]. However, several recent reports clearly demonstrate the high quality of the latest -omics datasets (e.g. [11,16,17]). In addition, many initiatives are ongoing to produce 'universal' annotation of data available in databases (reviewed in [14]). Hence, the technical requirements for the application of systems biology towards the definition of the molecular chain of events involved in VD appear to be met.

To furnish the enormous challenge of applying systems biology towards vascular disease with goals that can actually be reached, we suggest focusing on specific hallmarks of VD to be studied with adapted -omics tools and the resulting data to be exploited in the iterative cycle of systems biology. Disparate data support the hypothesis that disturbance in the homeostasis of the extracellular matrix (ECM) is among the main and early significant changes in both micro- and macrovascular complications, but systematic analysis has never been performed to clearly pinpoint the modifications of the ECM at an early stage in VD [18]. ECM remodelling is currently not specifically addressed using the standard -omics techniques. As we anticipate that

this is a key event in VD, we suggest to obtain additional -omics data on ECM remodelling by new large-scale techniques using proteolytic signature peptides or specific isotope labelling followed by mass spectrometry to identify protease substrates [19]. Another issue we believe to be of importance are VD-predisposing factors associated with human ageing and accelerated telomere shortening [20]. There is increasing evidence that telomere dysfunction and DNA damage accumulation contributes to the development of VD [21,22]. Thus, instead of focusing on individual genes involved in telomere dysfunction, we propose to perform -omics screening of animal models of telomere shortening [22] to gain additional input in the systems biology-based models of VD.

The results of these efforts should have a major impact on the VD clinical management:

(a) Computational models of the VD-associated (patho) physiological changes are expected to be established. These models, along with data-driven statistical models, may allow us to display the timely correlation of relevant pathophysiological events. As a consequence, valid disease biomarkers and biomarker panels may be identified for early disease detection when no clinically relevant events have yet been registered. Furthermore, these models and biomarkers may enable prognosis of disease development and facilitate initiation of appropriate (pre-emptive) therapeutic measures that prevent or delay development of clinically relevant disease. These developments, if successful, will subsequently enable stratification of patients based on prognostic biomarkers and the establishment of valid mediators as surrogate end points, resulting in shorter clinical trials that reach statistical significance based on a lower number of participants.

(b) The establishment of an algorithm (similar to the Framingham score, [23]) is further expected. This will be based on molecular and classical clinical and laboratory data alike, and will enable the non-invasive assessment and prognosis of development of vascular disease with much higher accuracy than currently possible.

(c) Another major result based on the knowledge of molecular changes and networks of these in the development and progression of micro- and macrovascular diseases will be the discovery of new, more appropriate potential targets for individual targeted therapeutic interventions. Upon validation of this newly gathered information in animal and cell culture models, interference studies may be performed aiming to determine whether interference/intervention does influence disease and test whether the identified molecular structures represent therapeutic targets. The new validated biomarkers and animal models may result in improvements in clinical intervention trials by enabling preclinical testing in relevant animal models based on biomarkers relevant to human disease. In parallel analysis, the changes in proteome/metabolome (and transcriptome, if applicable) that are induced by the interfering agents will enable the development of useful surrogate markers for clinical end points.

Based on these thoughts, it appears that a systems biology-driven approach, as a concerted effort of clinicians, biologists and systems biologists, is being initiated to battle vascular disease. Such a holistic approach integrating massive molecular, clinical and pathological data in combination with novel biostatistical, informatics/computational and bioinformatics approaches will most likely have a major impact on the management of VD and will pave the way for individualized targeted therapy.

Conflict of interest statement. None declared.

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Disease classification: a pitfall of the ERA/EDTA registry?

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Introduction

Monoclonal proliferations of the B-cell lineage, often referred to as plasma cell dyscrasias or plasma cell disorders, are characterized by abnormal, uncontrolled expansion of a single clone of B cells at different maturation stages, with a variable degree of differentiation to immunoglobulin (Ig)-secreting plasma cells. They are thus usually associated with the production and secretion in blood of a monoclonal Ig or a fragment thereof. An ominous consequence of the secretion of monoclonal Ig products is their deposition in tissues, particularly in the kidney. These proteinaceous deposits can take the form of casts (in multiple myeloma cast nephropathy), crystals (in plasma cell disorder-associated Fanconi syndrome), fibrils (in light-chain and exceptional heavy-chain amyloidosis) or granular precipitates (in monoclonal immunoglobulin deposition disease). In a large proportion of patients, major clinical manifestations and mortality are related to visceral Ig deposition rather than to expansion of the B-cell clone. Indeed, except for multiple myeloma cast nephropathy, which is generally associated with a malignancy with a large tumour mass, Ig precipitation or deposition diseases frequently occur in the course of a benign B-cell proliferation or of a low-grade multiple myeloma.

Multiple myeloma is suggested to be one of the most common malignancies causing end-stage renal disease (ESRD), apart from neoplastic obstruction of the urinary tract. Until the 1980s, multiple myeloma-induced renal failure was associated with a very poor prognosis [1], and the question of the usefulness of dialysis had even been raised [2]. In recent years, both a better understanding of the triggering factors of acute renal failure and improvement of chemotherapy have prompted a reconsideration of the prognosis of acute kidney injury in multiple myeloma. In the majority of patients, renal function improves after correction of precipitating factors causing renal failure, namely hypercalcaemia, dehydration, infection and discontinuation of nephrotoxic drugs. However, a significant proportion of the patients who do not recover will require long-term renal replacement therapy, and uncertainties remain as to the outcome of those patients.

In this issue of *Nephrology, Dialysis and Transplantation*, Tsakiris and colleagues have attempted to address this question by using the ERA–EDTA Registry [3]. There is only one previous report from the United States Renal Data System (USRDS) that described characteristics and outcome of multiple myeloma patients in a national sample of patients with ESRD [4]. In both registries, the terms 'multiple myeloma' and 'light-chain associated nephropathy' (USRDS) or 'light-chain deposition disease' (LCDD) (ERA–EDTA Registry PRD code 82) are considered together although these terms designate completely different entities. In the ERA–EDTA Registry, there are separate PRD codes for amyloid (code 83) and Waldenström's disease (code 78).

Tsakiris and colleagues [3] conclude that the incidence of renal replacement therapy for ESRD due to multiple