Urinary peptidomics provides a noninvasive humanized readout of diabetic nephropathy in mice

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Nephropathy is among the most frequent complications of diabetes and the leading cause of end-stage renal disease. Despite the success of novel drugs in animal models, the majority of the subsequent clinical trials employing those drugs targeting diabetic nephropathy failed. This lack of translational value may in part be due to an inadequate comparability of human disease and animal models that often capture only a few aspects of disease. Here we overcome this limitation by developing a multimolecular noninvasive humanized readout of diabetic nephropathy based on urinary peptidomics. The disease-modified urinary peptides of 2 type 2 diabetic nephropathy mouse models were identified and compared with previously validated urinary peptide markers of diabetic nephropathy in humans to generate a classifier composed of 21 ortholog peptides. This classifier predicted the response to disease and treatment with inhibitors of the renin-angiotensin system in mice. The humanized classifier was significantly correlated with glomerular lesions. Using a human type 2 diabetic validation cohort of 207 patients, the classifier also distinguished between patients with and without diabetic nephropathy, and their response to renin-angiotensin system inhibition. Thus, a combination of multiple molecular features common to both human and murine disease could provide a significant change in translational drug discovery research in type 2 diabetic nephropathy.

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Diabetic nephropathy (DN) is among the most frequent complications of diabetes,[1] and recent evidence shows that kidney involvement is the main determinant of the increased risk of death in diabetic patients.[2] Despite significant advances in the understanding of the molecular mechanisms of type 2 diabetic (T2D, accounting for 90%–95% of the diabetes cases) DN, the current standard-of-care is, however, still based on achieving blood pressure control by use of inhibitors of the renin-angiotensin system (RASi) on top of glycemic control. While slowing down the progression of DN in most cases, this treatment does not halt the ineluctable progression to end-stage renal disease.[3] Therefore, new drugs specifically targeting the DN pathophysiology are needed to reduce the burden of this disease. The translation of the extensive knowledge and plethora of potential targets[4] obtained in animal models of DN and other diseases to the clinic has been extremely limited.[3,4] This has been attributed to the following: (i) human pathophysiology and drug susceptibility are often not adequately captured by animal models;[5,6] (ii) readouts used in preclinical studies are usually different from the required endpoints in clinical trials;[7] or (iii) those readouts are based on a single molecule or phenotypic trait that is not sufficient to assess pathology in detail and identify clinically valuable potential drug target (Figure 1a).[8,9] Ways have been developed to circumvent these concerns mainly in the field of cancer and immunology by the development of humanized mouse models.[10] An alternative to the development of these humanized animal models, which is technically complicated and not always feasible in the context of many complex diseases such as DN, might be the optimization of the experimental readout based on a panel of molecular features that are identical in both human and animal disease ignoring the nonhuman animal disease–related traits (Figure 1b).

Although a multitude of animal models of DN exist,[11] the most frequently used models of T2D DN are the BTBRob/ob

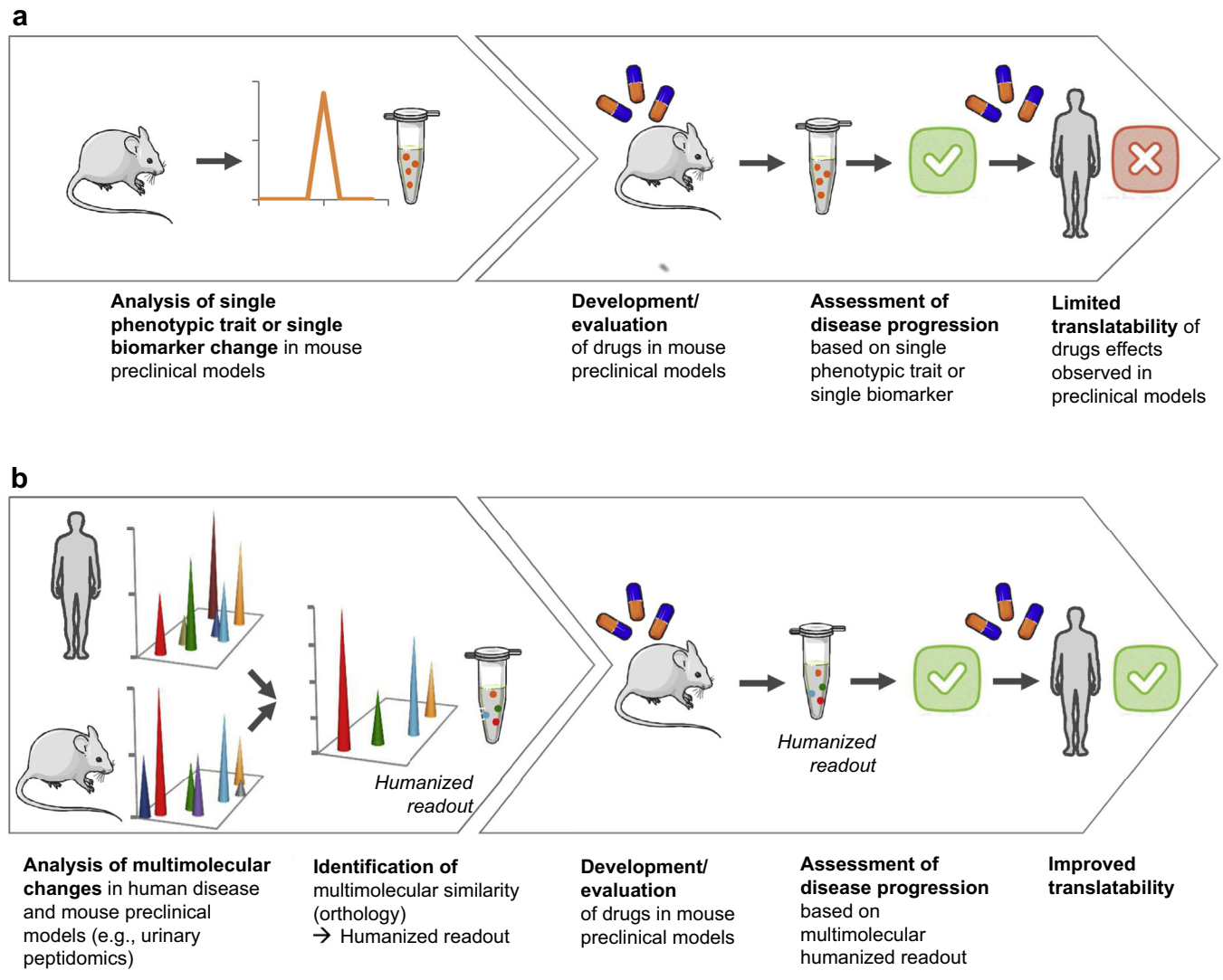

Figure 1 | Improving the translatability of animal models of disease by the development of a multimolecular humanized readout. (**a**) Disease readouts are often based on a single phenotypic trait or biomarker similar, if possible, in animals and humans. Such single feature does not capture the complexity of most disorders. Hence novel drugs, although successful in the preclinical phase based on such single feature readout in animal models, often fail in humans. (**b**) In contrast, the analysis of multimolecular changes using omics approaches, by, for example, urinary peptidomics, allows a more complete description of complex phenotypes. Furthermore, the combination of these multiple features similar (ortholog) in human disease and animal models, leading to a "humanized" readout, most likely will more efficiently translate the effects of new drugs in preclinical models to the clinic.

mice[12] and uninephrectomized db/db mice on a C57BLKS background (UNxdb/db).[11] These 2 models are obese insulin-resistant DN models due to leptin or leptin-receptor deficiency, respectively, displaying renal features similar to that observed in humans, including progressive albuminuria with advanced glomerular lesions and mild focal interstitial fibrosis. In addition, standard-of-care treatment using RASi confers renoprotection in these models.[13,14] However this resemblance to human DN relies on albuminuria and histology and often lacks any established molecular similarity; moreover, access to histological data requires animal sacrifice, which is costly, time-consuming, and does not allow for longitudinal follow-up.

In humans, current noninvasive readouts of progression of DN are based on the urinary albumin excretion rate (AER) or estimation of the glomerular filtration rate (eGFR) or both using serum creatinine. However, reduction of AER is not always correlated with complete reversal of all phenotypic and *in situ* changes observed in preclinical mouse models of DN.[13] Moreover, although it is routinely performed in humans, eGFR assessment in mice is of little comparative value, because in mice 35% to 50% of the creatinine is secreted and not attributable to the glomerular filtration.[15] Although methods for GFR measurement, based on fluorescent inulin clearance, have been developed for mice, these are still invasive (and cannot be repeated frequently),[16] and will not detect small changes in renal function.

We and others have shown that urine is a rich and noninvasive source of biomarkers of disease in humans.[17–19] Mass spectrometry–based analysis of the endogenous urinary