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Frau
Präsidentin des Nationalrates
Mag. Barbara Prammer
Parlament
1017 Wien

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zu 8826 /J

Wien, 9. August 2011

Die schriftliche parlamentarische Anfrage Nr. 8826/J-NR/2011 betreffend Stoffwechsel-„Fingerabdruck", die die Abgeordneten Rupert Doppler, Kolleginnen und Kollegen am 16. Juni 2011 an mich richteten, wird nach Einholung einer Stellungnahme der Medizinischen Universität Innsbruck wie folgt beantwortet:

Einleitend ist festzuhalten, dass die Untersuchungen zu Stoffwechselprodukten im Harn ein hoffnungsvolles Forschungsgebiet sind, da man sich aufgrund der Metabolitenprofile im Harn erhofft, neue Biomarker zu entdecken, die für bestimmte Erkrankungen von diagnostischer Relevanz sind. Ziel ist dabei eine frühzeitige Diagnostik, sodass rechtzeitig entgegengesteuert werden kann, lange bevor die Erkrankung unumkehrbar ist. Beispielhaft wären hier vor allem weit verbreitete Erkrankungen wie Diabetes mellitus und Nierenfunktionseinschränkungen zu nennen, die in der Häufigkeit mit dem Alter sehr deutlich ansteigen. Daneben ist aber auch die Kapazität der Entgiftungsfunktion durch die Niere zu erwähnen, die sicherlich durch Gene beeinflussbar ist. Das Auffinden dieser für den Stoffwechsel der Niere wichtigen Gene trägt in erster Linie zum besseren Verständnis der Funktionsweise der Niere sowie der damit zusammenhängenden Erkrankungen bei. Aufgrund dieses Verständnisses wird es dann Überlegungen geben, welche der Metaboliten potentiell als Parameter zur Diagnose und damit als Biomarker einer dieser Erkrankungen wie Diabetes mellitus oder Nierenfunktionseinschränkungen eingesetzt werden können. Dies ist zu vergleichen mit längst bekannten Parametern aus dem Blut, die zur Risikoabschätzung für Herzerkrankungen verwendet werden (z.B. Cholesterin mit seinen Subfraktionen oder natriuretische Peptide wie BNP).

Zu Frage 1:
Dies lässt sich momentan noch nicht genau absehen, da die gewonnenen Erkenntnisse erst in großen Gruppen von Patienten untersucht werden müssen. Sicherlich wird es dabei aber um Erkrankungen wie Diabetes mellitus und Nierenerkrankungen gehen, bei denen die Niere ja substanziell beteiligt ist. Weiters wird es sich dabei um die zahlreichen Stoffwechselerkrankungen handeln, bei denen die Niere aufgrund ihrer Entgiftungsfunktion mitbeteiligt ist.

DVR 0000167

Zu Frage 2:

Die bisherige Forschungs- und Studienergebnisse konzentrieren sich auf die Weiterentwicklung der Nachweismethode und die Überprüfung der Korrelation zwischen spezifischen Metaboliten und potentiellen genetischen Markern und erlauben daher noch keine Aussagen über die Risiken für bestimmte Erkrankungen. Aufgrund der Neuartigkeit und Vorläufigkeit der Ergebnisse lassen sich daher auch noch keine Aussagen über die Exaktheit der Risikoabschätzung machen.

Dies kann erst gesagt werden, wenn ausreichend klinisch-epidemiologische Daten erhoben worden sind. Dabei steht im Vordergrund, dass diese untersuchten Metaboliten bezüglich der Vorhersage von Erkrankungen besser sein müssen wie die bisher bereits bekannten Biomarker (siehe dazu auch den angeschlossenen Überblicksartikel „Emerging risk factors and markers of chronic kidney disease progression", in dem es z.B. um neue Biomarker für das Voranschreiten von Nierenfunktionseinschränkungen geht).

Zu Frage 3:

An der (Weiter)Entwicklung von labordiagnostischen Methoden arbeitet national und international eine Vielzahl von Forschungsgruppen, mit dem Ziel, zukünftig Risiken für Erkrankungen früher und besser abschätzen zu können. Neuere technische Entwicklungen werden es ermöglichen, deutlich mehr Metaboliten (aktuell bis zu 300 und mehr) im Hochdurchsatz zu untersuchen. Dabei wird versucht werden, für bestimmte schon vorhandene oder sich abzeichnende Erkrankungen spezifische Metaboliten-Profile zu identifizieren. Aufgrund dieser Ergebnisse könnten sich neue diagnostische Marker (Biomarker) ergeben. Weiters könnten durch das bessere Verständnis der Pathogenese der untersuchten Erkrankungen neue „drug targets" resultieren, die zur Entwicklung von neuen Medikamenten führen.

Der Bundesminister:

Beilage

Emerging risk factors and markers of chronic kidney disease progression

Florian Kronenberg

Abstract | Chronic kidney disease (CKD) is a common condition with an increasing prevalence. A number of comorbidities are associated with CKD and prognosis is poor, with many patients experiencing disease progression. Recognizing the factors associated with CKD progression enables high-risk patients to be identified and given more intensive treatment if necessary. The identification of new predictive markers might improve our understanding of the pathogenesis and progression of CKD. This Review discusses a number of emerging factors and markers for which epidemiological evidence from prospective studies indicates an association with progression of CKD. The following factors and markers are discussed: asymmetric dimethylarginine, factors involved in calcium–phosphate metabolism, adrenomedullin, A-type natriuretic peptide, *N*-terminal pro-brain natriuretic peptide, liver-type fatty acid binding protein, kidney injury molecule 1, neutrophil gelatinase-associated lipocalin, apolipoprotein A-IV, adiponectin and some recently identified genetic polymorphisms. Additional epidemiological and experimental data are required before these markers can be broadly used for the prediction of CKD progression and before the risk factors can be considered as potential drug targets in clinical interventional trials.

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Competing interests
The author, the Journal Editor S. Allison and the CME questions author C. P. Vega declare no competing interests.

Introduction

In 2003, data from the US population-based Third National Health and Nutrition Examination Survey (NHANES III) reported that the total prevalence of chronic kidney disease (CKD) in the US adult population was 11%.[1] When patients were subdivided into the five stages of CKD according to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDOQI) guidelines, the prevalences of stages 1 through 5 CKD were 3.3%, 3.0%, 4.3%, 0.2% and 0.2%, respectively.[1] In Europe, the prevalence of CKD is very similar to that in the US,[2] but the prevalence is higher in Asia and Australia.[3] Disturbingly, the prevalence of CKD continues to increase, with NHANES data showing that CKD prevalence increased by 30% between 1990 and 2000.[4]

The incidence of end-stage renal disease (ESRD) varies widely between countries, with incidences of 100–150 per million population per year in Europe, 300 per million population per year in the US and Mexico and 400 per million population per year in Taiwan.[3] Many risk factors and markers for the development and progression of CKD exist (Figure 1) and these differ markedly between countries. Cost of treatment for ESRD is a major concern, particularly in light of the increasing prevalence of CKD. Although the prevalence of ESRD is only about 0.2%, ESRD programs now account for 6.7% of total Medicare expenditure and Medicare costs associated with ESRD increased by 57% between 1999 and 2004.[5]

This Review discusses a number of emerging factors and markers for which epidemiological evidence from prospective studies in humans and experimental data has

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Key points

- Chronic kidney disease (CKD) is a highly prevalent health problem with an increasing incidence and a strong tendency for progression
- Few studies have searched for emerging risk factors or markers for progression of CKD
- Available studies have identified markers related to nitric oxide synthesis, calcium–phosphate metabolism, natriuretic peptides, apolipoprotein A-IV, adiponectin and other parameters
- Further epidemiological and experimental studies are required to determine whether these factors are involved in the pathogenesis of CKD progression or whether they are just markers of the risk for disease progression
- Hypothesis-free approaches such as genome-wide association studies, metabolomic studies and other '-omics' technologies will identify new risk factors and markers of CKD progression in well-defined cohorts

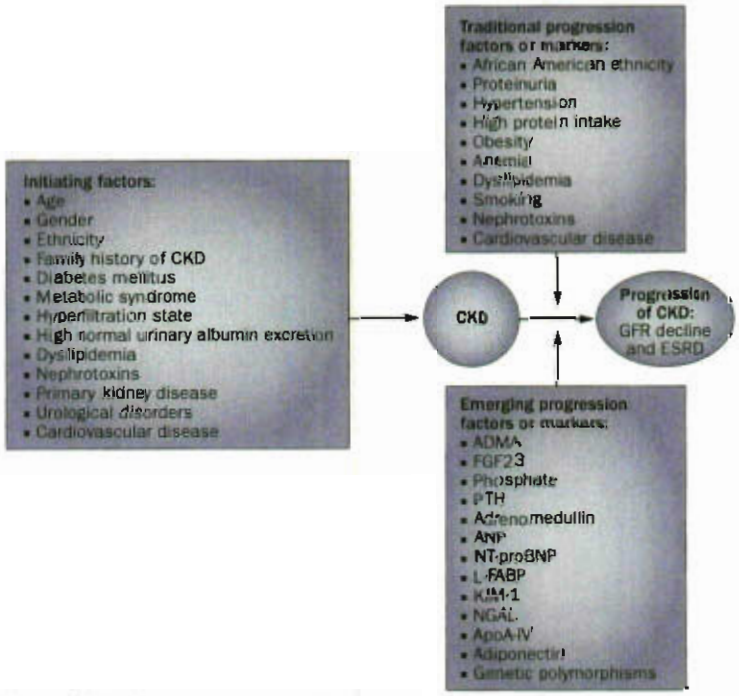

Figure 1 | Risk factors and markers for the initiation and progression of chronic kidney disease. Traditional factors involved in the initiation and progression of CKD are discussed elsewhere.[37] The present Review discusses emerging risk factors and markers—such as ADMA, FGF23, ANP and NT-proBNP—that are shown in the bottom panel and require further study. Abbreviations: ADMA, asymmetric dimethylarginine; ANP, A-type natriuretic peptide; apoA-IV, apolipoprotein A-IV; CKD, chronic kidney disease; ESRD, end-stage renal disease; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; PTH, parathyroid hormone; KIM-1, kidney injury molecule 1; L-FABP, liver-type fatty acid binding protein; NGAL, neutrophil gelatinase-associated lipocalin; NT-proBNP, *N*-terminal pro-brain natriuretic peptide.

shown an association with CKD progression. Two main reasons exist for the interest in identifying new markers of CKD progression. Firstly, new markers will improve prediction of which patients are at risk of experiencing CKD progression and the associated consequences (for example, cardiovascular risk, comorbidities, requirement for renal replacement therapy, decline in quality of life and risk of death). Although a measurement of baseline kidney function is relatively easy to obtain, this parameter has limited predictive precision. Some parameters measured at baseline have a similar area under the curve (AUC) to kidney function measures when receiver operating characteristic (ROC) analysis is performed. This finding suggests that the discrimination capability of these parameters is similar to that of kidney function measures, indicating that these parameters could be surrogate parameters for kidney function and could be used to predict CKD progression alongside baseline glomerular filtration rate (GFR). The second main reason for the interest in the field is that additional markers might lead to a better understanding of the pathogenesis and progression of CKD, as in some cases, only small amounts of CKD progression can be explained after adjustment for baseline GFR.

At present, it is unclear whether many of the biomarkers are risk markers or risk factors for CKD progression. Risk markers are not causally involved in CKD progression but as they indicate the probability of progression, they might be useful diagnostic tools. Risk factors causally affect disease progression and are, therefore, interesting therapeutic targets. Of course, epidemiological observations do not necessarily prove causality. Such observations merely generate hypotheses that need to be proven in animal models, and might eventually result in interventional methods that slow or prevent progression of CKD. Further epidemiological data and experimental studies are needed before many of the emerging risk markers and factors can be used to predict CKD progression or be considered as potential drug targets in clinical interventional trials, and many parameters will not reach clinical application.

Definition of CKD progression

No clear and uniform definition exists for the progression of CKD, and the choice of definition is usually influenced by the cohort under observation and the duration of the prospective observation period (Table 1). Typical end points are surrogates of the GFR slope (for example, doubling of baseline serum creatinine level and/or the need for renal replacement therapy, a particular relative increase from baseline serum creatinine level, yearly or monthly decline in GFR, GFR reduction to 50% of baseline, dialysis or kidney transplantation, or graft loss) or particular indices of renal damage (for example, worsening of proteinuria, or appearance of albuminuria in patients with diabetes). This lack of uniformity is a well-known limitation when comparing the prognostic potential of putative risk factors and markers.

Risk factors and markers of CKD progression

This Review focuses on possible risk factors and markers of CKD progression for which at least one prospective study with a sample size of at least about 100 individuals has been performed. Rather than describing studies of classical or established risk factors such as age, gender, blood pressure, smoking, metabolic syndrome, uric

Table 1 | Prospective observational studies investigating emerging risk factors and markers for CKD progression*

Study	Study population	End points	Duration of follow-up	Parameters associated with CKD progression	Parameters showing no association with CKD progression
MMKD study[15,25,65,101,111,112]	177 nondiabetic patients with primary CKD	Doubling of baseline serum creatinine and/or renal replacement therapy (n=65)	Up to 7 years; median 53 months	ADMA, FGF23, Ph, Ca–Ph, PTH, adiponectin (in men), apoA-IV, ANP, adrenomedullin, NT-proBNP (borderline)	Ca
Hanai *et al.*[18]	225 patients with type 2 diabetes	Progression of diabetic nephropathy (n=37)	Median 5.2 years	ADMA	—
EURAGEDIC case–control study[17]	397 patients with type 1 diabetes with nephropathy	Dialysis or kidney transplantation (n=70); yearly decline of GFR	Median 11.3 years	ADMA	—
Ravani *et al.*[16]	131 newly referred patients with stages 2–5 CKD	Dialysis treatment or GFR reduction to half of baseline (n=29)	Mean 27 months	ADMA	—
Schwarz *et al.*[23]	985 US male veterans with stages 1–5 CKD	Doubling of baseline creatinine and/or renal replacement therapy (n=258)	Median 2.1 years	Ph, Ca–Ph	Ca
AASK[24,113]	1,094 black patients with hypertensive nephrosclerosis (GFR 20–65 ml/min/1.73 m²)	Decline in GFR by either 50% or 25 ml/min/1.73 m² from baseline or occurrence of ESRD	Median 4 years	Ph	Ca–Ph, NT-proBNP
Voormolen *et al.*[26]	448 patients with stages 4–5 CKD and GFR <20 ml/min/1.73 m²	Monthly decline in eGFR	Median 337 days	Ph, Ca–Ph	Ca
Levin *et al.*[27]	4,231 individuals with eGFR <30 ml/min/1.73m² in provincial CKD registry	Moderate progression: 2.3–5.0 ml/min/year (24%) Rapid progression: >5.0 ml/min/year (26%)	Median 31 months	Moderate progression: PTH Rapid progression: PTH, Ph, low Ca	—
EURAGEDIC case–control study[28]	198 patients with type 1 diabetes with nephropathy	Dialysis or kidney transplantation (n=40)	Median 8.1 years	Adiponectin	—
Finnish Diabetic Nephropathy Study[67]	1,330 patients with type 1 diabetes mellitus	Progression to ESRD (83/296 patients with macroalbuminuria progressed)	Mean 5 years	Adiponectin in individuals with macroalbuminuria	No association in individuals with normoalbuminuria or microalbuminuria
Ravani *et al.*[30]	168 newly referred patients with stages 2–5 CKD	Dialysis treatment (n=48)	Median 57 months	Vitamin D	—
Bolignano *et al.*[81]	96 patients with stages 2–4 CKD	Doubling of baseline serum creatinine and/or renal replacement therapy (n=31)	Median 18.5 months	Urinary and serum NGAL	—
van Timmeren *et al.*[82]	145 renal transplant recipients	Graft loss (n=31)	4 years (range 3.2–4.5 years)	Urinary KIM-1	—

*Small studies with sample sizes much below 100 are not included. Abbreviations: AASK, African American Study of Kidney Disease and Hypertension; ADMA, asymmetric dimethylarginine; ANP, A-type natriuretic peptide; apoA-IV, apolipoprotein A-IV; Ca, calcium; Ca–Ph, calcium–phosphate product; CKD, chronic kidney disease; EURAGEDIC, European irrational approach for the genetics of diabetic complications; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; KIM-1, kidney injury molecule 1; MMKD, Mild to Moderate Kidney Disease; NGAL, neutrophil gelatinase-associated lipocalin; Ph, phosphate; PTH, parathyroid hormone.

acid, or commonly investigated parameters such as inflammation and hemostasis, emerging new parameters are discussed (Figure 1). Markers that have only been investigated in acute kidney injury (AKI) are not considered, and animal models and interventional studies are discussed only briefly.

Asymmetric dimethylarginine

Asymmetric dimethylarginine (ADMA) is a naturally occurring amino acid and an inhibitor of nitric oxide (NO) synthase. ADMA competes with arginine for binding sites on NO synthase and leads to decreased production of NO (Figure 2). Experimental data indicate that decreased local NO production might be involved in progressive kidney damage.[6,9]

ADMA is generated from the degradation of methylated proteins containing ADMA residues (Figure 2). It is metabolized mainly by dimethylarginine dimethylaminohydrolase (DDAH)[10] into dimethylamine and citrulline, and only a small fraction of ADMA is excreted unchanged by the kidney. As DDAH and NO synthase are co-localized in glomerular endothelial cells and

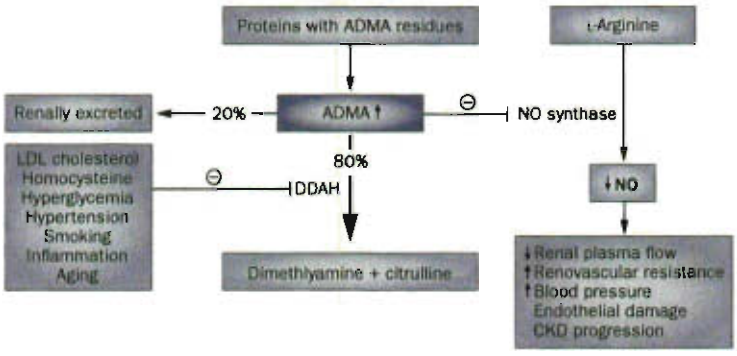

Figure 2 | The role of ADMA in the progression of kidney disease. ADMA is mainly metabolized by the enzyme DDAH. The activity of DDAH is strongly impaired in kidney disease, which leads to increased ADMA levels. These increased ADMA levels inhibit NO synthase, thereby resulting in decreased NO levels, which has major adverse consequences for the kidney and might lead to progression of CKD. Abbreviations: ADMA, asymmetric dimethylarginine; CKD, chronic kidney disease; DDAH, dimethylarginine dimethylaminohydrolase; NO, nitric oxide.

tubular cells, even mild kidney dysfunction leads to an increase in ADMA concentration.[11] These observations are in line with findings from metabolic studies demonstrating that the kidney is the main site at which ADMA is removed from the circulation.[12]

Conditions such as hypercholesterolemia or hyperglycemia inhibit DDAH, which results in increased ADMA levels (Figure 2). As the resulting decrease in NO production is associated with decreased renal plasma flow, increased renovascular resistance, hypertension and endothelial damage, ADMA is an interesting potential marker for cardiovascular disease.[13]

ADMA levels are markedly elevated in renal impairment.[11,14,15] Together with the experimental evidence showing ADMA to be a CKD progression factor in animals,[6–9] four prospective studies have found an association between ADMA level and CKD progression in humans (Table 1). The Mild to Moderate Kidney Disease (MMKD) study, which followed 177 patients with primary nondiabetic CKD for up to 7 years, showed that each 0.1 µmol/l increase in ADMA level was associated with a 47% increase in the probability for progression of CKD (Table 1), after correction for baseline serum creatinine levels.[15] Ravani and colleagues found that high ADMA levels were associated with an increased risk of ESRD and mortality among 131 patients with CKD.[16] A study in 397 patients with type 1 diabetes and overt diabetic nephropathy showed that an ADMA level higher than the median was associated with a faster yearly decline in GFR and a greater risk of developing ESRD over a median follow-up of 11.3 years than an ADMA level below the median.[17] Adjustment for baseline variables (including GFR) weakened the association but it remained borderline significant ($P = 0.055$). A study including 225 patients with type 2 diabetes (183 with normoalbuminuria and 42 with microalbuminuria) followed up over 5.2 years reported progression of diabetic nephropathy to a more advanced stage in 37 patients.[18] Patients with ADMA levels above the median had a 2.7-fold higher risk of disease progression than patients with ADMA levels below the median. Together, these studies provide strong evidence that increased ADMA levels are associated with progression of CKD.[15–18]

Experimental data in a rat remnant kidney model indicate a causative role for ADMA as a progression factor for kidney disease, suggesting that lowering ADMA might be beneficial.[19,20] Overexpression of DDAH significantly decreased ADMA levels and prevented the progression of renal dysfunction by inhibiting the progressive loss of glomerular and peritubular capillaries, interstitial fibrosis and urinary protein excretion. Furthermore, overexpression of DDAH suppressed the enhanced expression of TGF-β (which might have been a consequence of hypoxic conditions resulting from the loss of glomerular and tubular capillaries). Similar findings were reported in a rat model of diabetic nephropathy.[21] Administration of DDAH protein or enhancing its activity was therefore suggested as a potential therapeutic strategy for the treatment of CKD. In this context, a long-term, randomized, controlled trial in obese patients with CKD is of interest: patients received a low-protein diet supplemented with either keto-amino acids or placebo for 3 years.[22] Individuals who received keto-amino acids experienced pronounced decreases in ADMA levels, visceral body fat and proteinuria, and a significant delay in the progression of CKD.

Ca–Ph metabolism, vitamin D and FGF23

Disturbances in calcium–phosphate metabolism affect not only cardiovascular morbidity and mortality in patients with CKD, but also influence the progression of CKD.

Ca, Ph, Ca–Ph product and PTH

Although parameters of calcium–phosphate metabolism such as calcium, phosphate, calcium–phosphate product and parathyroid hormone (PTH) are some of the most comprehensively monitored parameters in patients with CKD, only a few studies have investigated whether one or more of these parameters is associated with CKD progression (Table 1).[23–27] The available studies found an increasing risk of progression with increasing phosphate concentrations.[23–27] These data are in line with clinical observations showing that phosphate restriction in patients with CKD who do not yet require renal replacement therapy might help to preserve residual kidney function[28] and that phosphate binders prevent progression of CKD.[29,30]

Three observational studies found an association between calcium–phosphate product and the progression of CKD.[25,25,26] Four studies showed no clear association between calcium concentrations and progression of CKD,[23,25–27] but two studies found an association between high PTH concentrations and CKD progression.[25,27] Furthermore, a retrospective cohort study examined

insurance claims from 186 and 517 predialysis adults with CKD and diabetes, with and without secondary hyperparathyroidism, and found that individuals with secondary hyperparathyroidism had an almost sevenfold increased risk of requiring dialysis treatment during the 6-year follow-up period compared with those without secondary hyperparathyroidism.[31]

Early experimental work suggested that phosphate restriction has a PTH-independent beneficial effect on the progression of renal disease in rats.[32] These findings are limited by the fact that rats generally have a physiologic hyperphosphatemia. A role for PTH in CKD progression has been supported by little direct experimental or clinical evidence,[33] but experimental studies have shown that progression of renal failure is significantly attenuated by the administration of calcimimetics or parathyroidectomy.[34]

Vitamin D

In the general population, vitamin D deficiency is clearly associated with conditions such as hypertension, cardiovascular disease, diabetes mellitus, heart failure and cancer.[35] Despite the tremendous influence of vitamin D on health and even though vitamin D has a pronounced effect on calcium–phosphate metabolism, only one study has investigated the association between vitamin D and the progression of CKD (Table 1).[36] This study followed 168 consecutive patients newly referred to a CKD clinic for an average of 48 months. During this time, 48 patients started dialysis treatment and 78 patients died. Level of 25-hydroxyvitamin D was an independent inverse predictor of progression to ESRD or death even after adjustment for age, estimated GFR (eGFR), heart failure, and other variables. An increase of 25 nmol/l (10 ng/ml) in 25-hydroxyvitamin D level was associated with a 40% decrease in the hazard ratio for CKD progression.[36] Interestingly, 25-hydroxyvitamin D was a better predictor of progression to ESRD or death than was 1,25-dihydroxyvitamin D.

These findings in humans are supported by solid evidence from animal studies. Although vitamin D therapy was previously assumed to be nephrotoxic, the observed nephrotoxic effects were probably a result of vitamin D-induced hypercalcemia in patients with CKD.[37] Recent experimental evidence has clearly shown that vitamin D and its analogues attenuate progression of CKD in various animal models.[33,38,39]

Fibroblast growth factor 23

The MMKD study showed high plasma fibroblast growth factor 23 (FGF23) concentrations to be one of the strongest risk factors or markers of CKD progression (Table 1).[25] FGF23 is a phosphatonin thought to be involved in the systemic balance of phosphate levels through interactions with the intestines, bone and kidneys.[40,41] In the clinical setting, elevated levels of FGF23 have been shown to be associated with hypophosphatemia, low 1,25-dihydroxyvitamin D levels and osteomalacia.[42,43] Experimental administration of FGF23 to animals produces similar disturbances in calcium–phosphate metabolism, and inactivation of the *Fgf23* gene in mice results in hyperphosphatemia and high circulating 1,25-dihydroxyvitamin D levels.[44,45] Under physiological conditions, hyperphosphatemia owing to a dietary phosphate load stimulates the secretion of FGF23, resulting in phosphaturia and suppression of the renal production of vitamin D.[46] In the presence of renal impairment, the decrease in renal function is paralleled by increases in FGF23, serum phosphate and PTH concentrations across a wide range of GFRs.[25,47] In advanced stages of CKD, disturbances in this regulatory network result in very high serum levels of FGF23.[48] However, the clinical studies could not determine whether the increased FGF23 levels are the cause of the increased serum calcium and phosphate levels or their consequence. Experimental studies showed that the increase in FGF23 levels preceded the decrease in serum 1,25-dihydroxyvitamin D concentrations, suggesting that FGF23 has an important role in the development of secondary hyperparathyroidism.[49] This idea is supported by results from clinical studies that found that serum levels of FGF23 increased in the early stages of CKD, even before serum phosphate and calcium concentrations had become abnormal.[50] Although it is still not clear whether increased FGF23 levels are a cause or a consequence of disturbances in mineral metabolism in individuals with CKD, FGF23 is an excellent indicator of the complex disturbances of calcium–phosphate metabolism induced by CKD, and the resulting consequences of the disturbed mineral metabolism that are accompanied by vascular calcification.[51–53] Of interest, a study in uremic rats published in 2009 showed that FGF23 prevented the progression of kidney disease but aggravated renal osteodystrophy by decreasing 1α-hydroxylase level and thereby lowering vitamin D level.[54] The authors speculated that if the FGF23 signaling pathway could be separated into a phosphaturic pathway and a 1α-hydroxylase pathway, the phosphaturic pathway would be an ideal drug target. Interestingly, studies in predialysis CKD demonstrated that increasing FGF23 levels are independently associated with left ventricular mass index and left ventricular hypertrophy.[55] In patients commencing hemodialysis treatment, increased FGF23 concentrations were independently associated with an increased risk of mortality during the first year of dialysis treatment.[56]

Only one prospective observational study in humans has investigated the association between CKD progression and levels of FGF23 together with levels of other parameters of calcium–phosphate metabolism.[25] Although FGF23, calcium–phosphate product, phosphate and PTH levels predicted progression of CKD as single variables (after adjustment for age, gender, GFR and proteinuria), the latter three parameters lost their predictive value when FGF23 was added to the prediction model.

Adiponectin

Adiponectin is a major adipocyte secretory protein that improves insulin sensitivity and possesses anti-inflammatory and anti-atherosclerotic properties.[57] Low adiponectin levels are associated with insulin resistance,[58,59] obesity and other features of the metabolic syndrome[60] and also with type 2 diabetes mellitus and cardiovascular disease.[61–63] As insulin resistance is already present in individuals who have a mild degree of renal impairment and also in patients who have primary CKD and a normal GFR,[64] an association between low adiponectin levels and progression of CKD would be expected. Surprisingly, however, three independent studies have shown an association between high adiponectin levels and CKD progression (Table 1).[65–67] The MMKD study found that high adiponectin level was an independent predictor of disease progression in men but not in women.[65] Jorsal and colleagues followed 198 patients with type 1 diabetes mellitus and diabetic nephropathy for an average of 8 years.[66] The 40 patients who reached ESRD during the observation period had significantly higher adiponectin levels at baseline than those who did not. An adiponectin level above the median was associated with a 2.7-fold higher GFR-adjusted risk of ESRD than a level below the median. Similar results were observed in a subgroup of 296 patients with type 1 diabetes mellitus and macroalbuminuria of the Finnish Diabetic Nephropathy Study.[67] Adiponectin levels were significantly higher among the 83 patients who progressed to ESRD than among those who did not progress. Adiponectin was not significantly predictive of progression to ESRD in patients with normoalbuminuria or microalbuminuria.

A number of hypotheses for these unexpected findings have been proposed. One suggestion is that the increased adiponectin level is associated with a change in the ligand–receptor reactivity (as occurs with other hormone–receptor systems in renal failure).[68] Other suggestions include the idea that the increased adiponectin level in CKD is a result of reduced adiponectin clearance by the kidney[69] or that it is a counter-regulatory response to metabolic derangements in renal failure.[70] Interestingly, experimental data from 2009 suggest that elevated plasma adiponectin levels in patients with CKD might play a compensatory role that aims to counteract renal dysfunction associated with mesangial cell disorders.[71] Resistance to adiponectin[72,73] is also a possibility, either as a result of adiponectin dysfunction or owing to the dysfunction or downregulation of adiponectin receptors with a counter-regulatory increase in adiponectin secretion. Several prospective studies have failed to find an association between low adiponectin levels and cardiovascular outcomes or death, particularly in individuals who already have severe and chronic disease at baseline. The Ludwigshafen Risk and Cardiovascular Health (LURIC) study, which followed almost 2,500 patients who had coronary artery disease at baseline for more than 5 years, found that increased adiponectin levels were strongly associated with all-cause and cardiovascular mortality.[74] The Modification of Diet in Renal Disease (MDRD) study reported similar findings in patients with a mean baseline GFR of 33 ml/min/1.73 m^2.[75] Kistorp *et al.* reported that high adiponectin levels were predictive of mortality in patients with chronic heart failure, independently of the severity of their disease.[76] These studies invite speculation that the upregulation of adiponectin in chronic diseases is a compensatory attempt to attenuate endothelial and vascular damage.[77]

Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a small (25 kDa) protein that is expressed in renal tubular cells and released into the blood and urine after exposure to harmful stimuli such as ischemia or toxicity. The release of NGAL occurs within hours after the stimulus and long before an increase in serum creatinine level occurs. NGAL level has therefore been proposed to be real-time indicator of active kidney damage whereas creatinine level and GFR are markers of functional nephron number.[78] The role of NGAL as a specific, sensitive and early predictor of AKI after contrast media administration, septic shock, kidney transplantation and cardiac surgery has been investigated extensively and has been reviewed elsewhere.[78,79]

Data indicate that NGAL is not only predictive for AKI but also for the progression of CKD. One study found that urinary NGAL levels were significantly higher in individuals with membranous nephropathy than in controls and that patients with high baseline urinary NGAL levels had a significantly higher risk of worsening residual renal function than those with low levels.[80] Another study in 96 patients with nonterminal stage 2–4 CKD found that both serum and urinary NGAL levels were directly associated with the progression of CKD over a median follow-up of 18.5 months, independently of age and GFR at baseline (Table 1).[81] A pilot study in 78 patients with CKD showed that baseline urine NGAL levels correlated strongly with changes in serum creatinine (coefficient of correlation $r = 0.77$) and GFR ($r = -0.40$) and that serum creatinine levels remained stable over an average follow-up period of 200 days in patients with low baseline urine NGAL levels.[82]

Two studies have described the potential of NGAL for monitoring the status and treatment response for various renal diseases. One of these showed that patients with nephrotic syndrome or interstitial nephritis had markedly elevated urinary NGAL levels that decreased in response to successful treatment.[83] The other study, in patients with severe proteinuria secondary to idiopathic membranous nephropathy, showed that a single high-dose bolus infusion of immunoglobulin was associated with a dramatic decrease in serum and urinary NGAL levels 1 h after infusion, a result that supports the anti-inflammatory properties of this intervention.[84] It remains to be seen whether NGAL will become a useful biomarker to quickly gauge a patient's response to a particular treatment.

Liver-type fatty acid binding protein

Clinical data on associations between liver-type fatty acid binding protein (L-FABP) and kidney disease are sparse. This protein is expressed in proximal tubular cells and increased expression and higher levels are seen in the urine of patients with kidney disease.[85,86] A large health screening program in more than 900 individuals revealed that average urinary levels of L-FABP were approximately 50% higher in patients with diabetes mellitus, hypertension or chronic hepatitis than in controls.[87] A study investigating L-FABP levels in patients with type 2 diabetes and different stages of nephropathy showed that urinary L-FABP was associated with the severity of diabetic nephropathy.[88] Small studies in patients with mild kidney dysfunction suggest that urinary L-FABP concentrations are increased in patients whose renal function deterioriates further.[86,89] Large studies with long-term follow-up are required to confirm these findings.

Kidney injury molecule 1

Kidney injury molecule 1 (KIM-1), a tubular transmembrane molecule with unknown function, is undetectable in healthy kidneys. As a phosphatidylserine receptor, KIM-1 seems to play an important role in apoptosis by recognizing apoptotic cells and directing them to lysosomes.[90] KIM-1 expression is increased in various renal diseases and is primarily expressed at the luminal side of dedifferentiated proximal tubules and in areas of fibrosis and inflammation.[91] Timmeren *et al.* found that levels of urinary KIM-1 were significantly elevated in patients with renal disease and undetectable in most healthy controls.[91] A prospective study in 145 renal transplant recipients followed for 4 years revealed that individuals with urinary KIM-1 concentrations in the second and third tertiles had a 3.6-fold and 5.1-fold increased risk, respectively, of losing the graft.[92] These results occurred independently of creatinine clearance, proteinuria and age of the graft donor. A post hoc analysis of a trial of patients with proteinuric kidney disease treated with various combinations of losartan, sodium restriction and hydrochlorothiazide to decrease proteinuria showed that the increased KIM-1 concentrations decreased in parallel with decreases in proteinuria even though kidney function did not change during the 6-week observation period.[93] Whether KIM-1 is just a sensitive biomarker for acute and chronic kidney disease or whether it is causally involved in kidney injury is unclear.

Apolipoprotein A-IV

Human apolipoprotein A-IV (apoA-IV) is a 46 kDa glycoprotein that is synthesized in intestinal enterocytes during fat absorption and incorporated into the surface of nascent chylomicrons (large lipoprotein particles that transport dietary lipids from the intestines to other locations in the body).[94] ApoA-IV has an important role in reverse cholesterol transport, which is disturbed in patients with CKD.[95] ApoA-IV is markedly elevated in ESRD[96,97] and is also a marker of kidney impairment that starts to increase in the earliest stages of CKD.[98] Immunohistochemical studies describing the immunoreactivity of apoA-IV in kidney tubular cells and investigations of apoA-IV in urine have shown that apoA-IV is filtered through the normal glomerulus and is mostly reabsorbed by proximal tubular cells.[99,100]

The MMKD study found that high apoA-IV levels are associated with CKD progression (Table 1). A 10 mg/dl increase in baseline apoA-IV (as usually occurs in patients with GFRs within the range 45–90 ml/min/1.73 m^2) was associated with an approximately 60% increased risk for progression of CKD during the median observation period of 53 months, independently of baseline GFR, proteinuria and other lipoprotein parameters.[101]

The association of higher apoA-IV concentrations with CKD progression was unexpected given the physiological functions of apoA-IV in reverse cholesterol transport and its reported antioxidative properties.[102] One might have anticipated that the increased apoA-IV levels in individuals with impaired kidney function would have resulted in increased removal of cholesterol from mesangial cells and this, together with the antioxidative properties apoA-IV, would lead to slower progression of CKD. Whether increased apoA-IV levels reflect an aspect of renal impairment that does not act in parallel with GFR or whether apoA-IV is not fully functional in cases of renal impairment is unclear. The latter hypothesis is an intriguing one, particularly in the light of the association of low apoA-IV levels with cardiovascular disease.[103,104] However, patients who had already experienced atherosclerotic events at the start of the MMKD study had lower apoA-IV levels than those without atherosclerotic complications in each stratum of GFR.[98]

Natriuretic peptides

Over recent years, the interaction of the heart and the kidneys became a focus of interest and the term 'cardiorenal syndrome' was coined. However, this term implies that the falling heart causes secondary impairment of kidney function. As patients with primary CKD develop cardiac symptoms in the short or long term, it became obvious that an interplay between the two organs occurs rather than a one-way street between the heart and kidney. This idea has resulted in a proposal for a new classification of the cardiorenal syndrome that better considers the functional and pathophysiological aspects, as thoroughly discussed in another review.[105] Many markers for cardiac dysfunction are also predictors of CKD progression. Prime examples are natriuretic peptides such as A-type natriuretic peptide (ANP), adrenomedullin and B-type natriuretic peptide (BNP). These peptides are potent hypotensive, diuretic, and natriuretic peptides that are involved in maintaining cardiovascular and renal homeostasis.[106–108] Increased plasma levels of ANP and adrenomedullin have been reported in individuals with cardiovascular disease, left ventricular dysfunction and in those with kidney disease.[109,110]

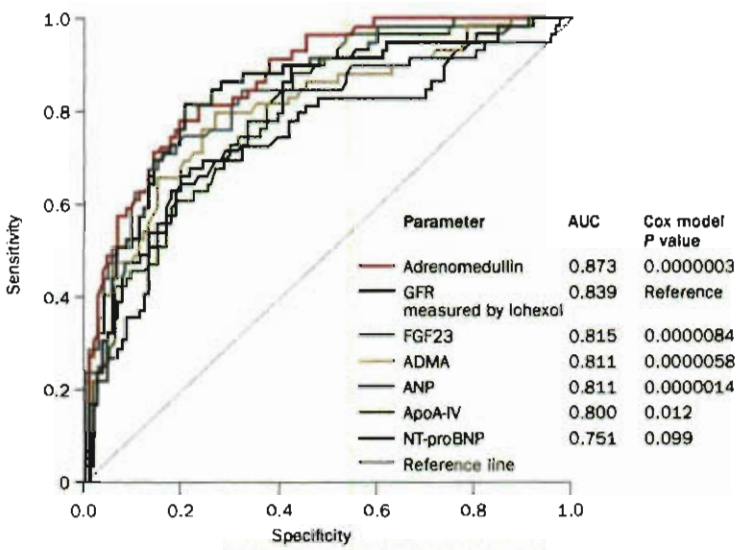

Figure 3 | Receiver operating curves from the MMKD study for GFR measured by Iohexol, ADMA, FGF23, apoA-IV and the natriuretic peptides ANP, NT-proBNP and adrenomedullin to predict progression of CKD. Area under the curve values for each of these parameters are shown as are *P* values for the addition of each of the particular variables to a Cox reference model adjusted for age, sex, proteinuria and GFR. Adrenomedullin showed the greatest area under the curve, which indicates that it had the best ability to discriminate between patients who would and would not experience CKD progression, even superior to the ability of GFR measured by Iohexol. Data are calculated from a number of references.[15,25,101,111,112] Abbreviations: ADMA, asymmetric dimethylarginine; ANP, A-type natriuretic peptide; apoA-IV, apolipoprotein A-IV; CKD, chronic kidney disease; FGF23, fibroblast growth factor 23; GFR, glomerular filtration rate; NT-proBNP; *N*-terminal pro-brain natriuretic peptide.

Adrenomedullin, ANP, and *N*-terminal pro-brain natriuretic peptide (NT-proBNP) levels were investigated in the MMKD study and high levels of each were found to be associated with CKD progression (Table 1).[111,112] The most pronounced association was found for adrenomedullin: an increase in this parameter by one standard deviation was associated with a 2.6-fold increased risk of disease progression. An increase in ANP by one standard deviation also more than doubled the risk of progression.[112] Interestingly, an independent and significant contribution of each parameter to the prediction of CKD progression was found when both parameters were added to the Cox regression model at the same time. NT-proBNP was only of borderline significance for predicting kidney disease progression in the MMKD study. In the African American Study of Kidney Disease and Hypertension (AASK), the incidence of renal disease progression increased with increasing NT-proBNP levels, but this finding was not significant after adjustment for kidney function (Table 1).[113] A small prospective study in 83 patients with CKD without clinical evidence of heart failure at baseline showed that average NT-proBNP levels at baseline were higher in patients who experienced progression of CKD than in those who did not.[114] However, no adjustment was made for baseline GFR levels in that study.

Comparison of parameters in the MMKD study

Most of the studies discussed above analyzed only one or few parameters at a time, making it virtually impossible to compare the results. As some of the parameters show strong correlations with each other, an analysis in a single Cox regression model must be performed with caution. Receiver operating curves for GFR, ADMA, FGF23, apoA-IV, ANP, NT-proBNP and adrenomedullin from the MMKD study indicate that adrenomedullin was best able to discriminate between patients with and without progression of CKD, with even better discriminatory ability than GFR measured by Iohexol (Figure 3). *P* values for the addition of each of the particular variables to a Cox regression model adjusted for age, sex, proteinuria and GFR show that adrenomedullin, FGF23, ADMA, ANP and apoA-IV significantly contributed to the prediction of CKD progression.[15,25,101,111,112] The same holds true for adiponectin, but only in men.[86] These parameters clearly contribute to the prediction of CKD progression and the associations of these variables with progression cannot simply be explained by correlations with GFR or proteinuria.

Genetic factors for progression of CKD

The major advantage of using genetic factors for monitoring CKD progression is their stability, as they are inherited at conception and do not generally change with disease. If they are found to be associated with a disease, they are often causally related to the disease in question.

Association studies using genetic polymorphisms

The perception of association studies of genetic polymorphisms has changed dramatically over the past few years. Previously, genetic variants were assumed to have a massive influence on complex phenotypes such as cardiovascular disease, diabetes mellitus and kidney disease. We now know, however, that polymorphisms with a relative risk of 2 or more for these complex phenotypes are very rare and such results in small studies might be false positive or chance findings. Genome-wide association studies and meta-analyses have shown that the genetic risk of a particular outcome associated with particular genetic variants is often only 1.2 or less. For example, about 20 genes with common variants are robustly associated with type 2 diabetes and show only moderate individual risk increases of between 1.1 and 1.4 on a per-allele basis.[115] Huge sample sizes are needed to obtain sufficient statistical power to identify disease-causing genetic variants.

Identifying a genetic polymorphism that is related to an outcome has the major advantage, however, that causality is likely. This is because—according to the rules of Mendelian randomization—genetic variants are not generally susceptible to the confounding that can distort findings in conventional observational studies, as long as assumptions such as random mating in the investigated population are fulfilled and if confounding by polymorphisms in linkage disequilibrium with the polymorphism under investigation can be excluded[116]

(Figure 4). This Mendelian randomization concept was applied in a recent follow-up study that investigated the association between adiponectin levels and progression of CKD in the EURAGEDIC study.[66] Adiponectin levels were associated with progression of type 1 diabetic nephropathy but no associations between several single nucleotide polymorphisms and progression of CKD were found. Given that only about 8% of the adiponectin level can be explained by variation within the adiponectin gene,[117] and as adiponectin levels explain only 10% of CKD progression at best, a very large number of samples would be required to identify an association of these variants with CKD progression (Figure 4). If in the future, a large study shows an association of genetic variants related to high adiponectin levels and an association of these variants with CKD progression, causality will be strongly suggested.

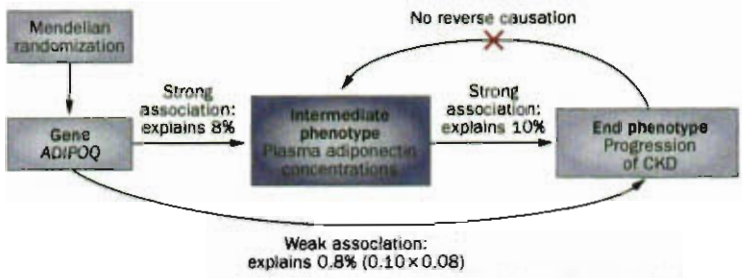

Figure 4 | Application of the concept of Mendelian randomization for identifying parameters causally related to progression of CKD, using adiponectin as an example. At conception, which of the two alleles is inherited from the mother and which of the two alleles is inherited from the father is determined randomly. Particular genetic variants within the *ADIPOQ* gene are associated with increased adiponectin levels; these variants explain about 8% of adiponectin concentration variance.[117] If high concentrations of adiponectin are truly associated with CKD progression, the genetic variants associated with life-long high adiponectin levels would be expected to lie more often within the group of patients who experience CKD progression. As this association is unlikely to be confounded by other factors, a reverse association can be excluded. Very large sample sizes are required to detect an association between a particular gene and progression of CKD if only small fractions (for example, below 10%) can explain the association between the gene and the intermediate phenotype and the association between the intermediate phenotype and CKD progression. This concept is discussed in more detail elsewhere.[116,138] Abbreviations: *ADIPOQ*, adiponectin gene; CKD, chronic kidney disease. Figure adapted from references 138 and 139 with kind permission of Springer Science + Business Media © Kronenberg, F. & Heid, I. M. *Medizinische Genetik* **19**, 304–308 (2007).

Genome-wide association studies

The introduction of genome-wide association studies provided a new and powerful approach in the quest for genes associated with complex phenotypes. These studies investigate hundreds of thousands of genetic variants across the entire human genome to determine whether they are associated with the phenotype of interest. In contrast to the hypothesis-driven approach of candidate gene studies, genome-wide association studies have the major advantage that they are hypothesis free and can therefore identify new candidate genes without making any biological assumptions. Owing to the large number of tests performed in these studies, however, large sample sizes are required.

A genome-wide association study of population-based cohorts with information on CKD, creatinine-based eGFR and cystatin-C-based eGFR in 19,877, 18,127 and 12,266 individuals, respectively, has identified several loci associated with kidney function.[118] Köttgen *et al.* found that uromodulin (*UMOD*) was associated with CKD and with creatinine-based eGFR. Furthermore, analysis of data from one of the studies—the prospective Atherosclerosis Risk in Communities (ARIC) study—identified a variant in this gene region that was associated with a decreased relative risk of incident CKD (hazard ratio 0.81, 95% CI 0.72–0.92, $P = 0.001$). Rare mutations in the *UMOD* gene are known to cause autosomal dominant diseases such as familial juvenile hyperuremic nephropathy, glomerulocystic kidney disease, and medullary cystic kidney disease type 2.[119–121] It is very interesting that such rare mutations that cause monogenic diseases can point to common variants in the same gene regions that influence disease-related phenotypes on the population level. Other genes identified to be associated with creatinine-based eGFR in the genome-wide association study reported by Köttgen *et al.* were shroom family member 3 (*SHROOM3*) on chromosome 4 and spermatogenesis associated 5-like 1 (*SPATA5L1*) at the *GATM-SPATA5L1* locus on chromosome 15.[118] In addition, stanniocalcin 1 (*STC1*) on chromosome 8 and an intergenic single nucleotide polymorphism (SNP) between the gene loci for cystatin C (*CST3*) and cystatin 9 (*CST9*) on chromosome 20 were found to be associated with cystatin-C-estimated eGFR. Loci such as *GATM-SPATA5L1* and genes in the cystatin superfamily gene cluster (*CST3* and *CST9*) are involved in creatinine and cystatin synthesis and do not necessarily reflect susceptibility for CKD or true GFR. Most of the newly identified genes need further functional characterization and association studies with further kidney-related phenotypes and with progression of CKD. Other gene loci previously thought to be associated with CKD or CKD progression (for example, angiotensin-I-converting enzyme, apolipoprotein E, methylenetetrahydrofolate reductase and transcription factor 7-like 2[122–125]) did not show genome-wide significance in Köttgen *et al.*'s study.

Köttgen *et al.* found that, together, the genetic loci identified to be associated with eGFR estimated by creatinine level explained about 0.7% of the variance of creatinine-estimated eGFR and that the genetic loci identified for eGFR estimated by cystatin explained about 3.2% of the cystatin-C-based eGFR variance.[118] Despite the high heritability of GFR, this small percentage is not surprising and is in line with findings from studies of other quantitative traits of similar heritability.[126–128] The finding indicates that other—not yet identified—genetic loci exist that influence kidney function and that many rare variants within the identified genes might explain the variance further.[129] Genome-wide association studies

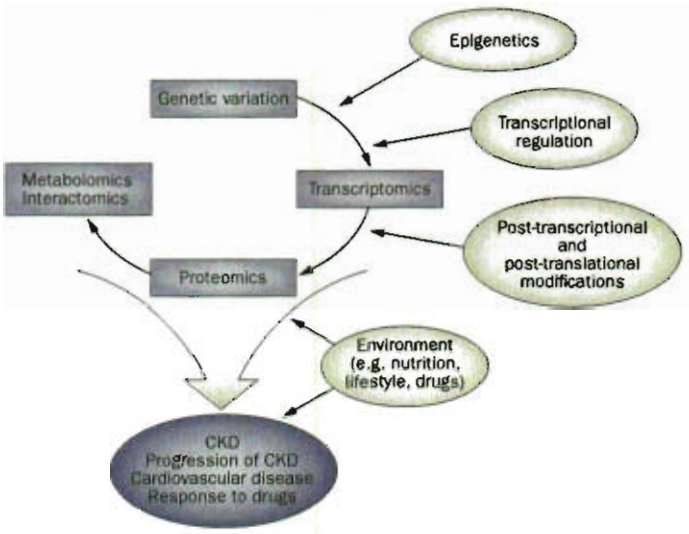

Figure 5 | The systems biology approach for identifying contributors to CKD, progression of CKD, cardiovascular outcomes and responses to drug treatment. Combining data on genetic variation, transcriptomics, proteomics and metabolomics, and considering various regulatory and modulatory influences as well as environmental interactions, will improve our understanding of the pathomechanisms of diseases. Abbreviation: CKD, chronic kidney disease.

and meta-analyses with sample sizes approaching 100,000 individuals are in progress and will identify new genes associated with CKD. Future collaborative studies will deal with the identification of genes associated with various forms of primary CKD as well as those associated with progression of CKD. Owing to the small size of studies of patients with primary CKD and prospective investigations on CKD progression, identifying genes for these extended phenotypes will be a real challenge.

The future

Several biochemical and genetic factors that have the potential to be markers of CKD progression or even factors involved in this process have been discussed. However, the findings discussed here are only a prelude. Various disciplines—many that have the suffix '-omics'—are breaking new ground (Figure 5). Metabolomics is the rapidly evolving field of measuring all endogenous metabolites in a cell or fluid such as plasma, serum, or urine.[130] Measuring intermediate phenotypes such as the small molecules that are involved in the pathway of a disease instead of simply classifying an individual as 'healthy' or 'diseased' might provide more detail about potentially affected pathways that relate more directly to the etiology of the disease. As all currently known metabolites can be measured in a single procedure or a few procedures, many new metabolites directly or indirectly involved in the development or progression of CKD would be identified. The same hypothesis-free approach has already been successfully applied in genome-wide association studies to identify genes and genetic variants involved in the development of complex diseases.[131,132] By combining genome-wide association studies with metabolomics, our group has found associations of common SNPs that are associated with the altered homeostasis of key metabolites in the human body and can explain up to 12% of the observed variance in levels of these metabolites.[133] When ratios of certain metabolite concentrations were used as a proxy for enzymatic activity, up to 28% of the observed variance could be explained. The combined approach of metabolomics and genome-wide association studies should provide insight into the pathomechanism of diseases and, in the future, the additional consideration of gene expression and proteomics in a combined analysis will substantially improve identification of risk factors for CKD and CKD progression. This systems biology approach might bring us closer to a personalized health-care system that will consider the functional basis for a response to a given drug treatment, nutritional intervention or environmental challenge.

The systems biology approach, however, will not only allow the combination of various '-omics' techniques but will require researchers to work together to obtain the sample size required for sufficient statistical power. In genetic epidemiology and genome-wide association studies, meta-analyses of 10,000 to 100,000 samples are routinely performed.[115,118,126–128,134] Similar numbers will be needed for this systems biology approach. In addition, sufficient standardization of analyses and procedures will be very important. Such standardization is already in place for genotyping and sequencing and efforts are underway to standardize creatinine measurements.[135] Although collaboration between study groups, which is necessary to attain the required sample sizes, was inconceivable a few years ago, it has now become standard practice in the field of genome-wide association studies.

Conclusions

The emerging parameters associated with CKD progression must be studied further to determine whether they are causally related to progression of CKD or whether they simply predict the probability of progression. The parameters might reflect diminished glomerular filtration, disturbances in tubular function or unknown contributors to kidney function that are unrelated to glomerular or tubular function. Some of the parameters are well-known markers of chronic heart failure, confirming the pronounced crosslinking between CKD and heart disease.

The evidence is still too sparse for most of these parameters to be recommended for broad clinical use in the diagnosis of CKD progression. Many parameters have only been investigated in a few studies, in small studies or in studies with differing definitions of CKD progression (Table 1). The results from animal models for some parameters are intriguing but application in humans needs to be demonstrated. Whether all markers for AKI will have the same value for diagnosis of CKD or CKD progression is unclear. Some parameters might be useful for the monitoring of therapies that influence kidney function (positively or negatively) and might reveal renal

changes long before they are identified by conventional measurements such as changes in GFR or proteinuria.

In the meantime, the usefulness of cohorts from large population-based studies such as the Framingham Heart Study, the ARIC study, the Cardiovascular Health Study and the Rotterdam Study for studying renal phenotypes has been demonstrated.[118,123,125] We await results from large prospective cohort studies—such as the Chronic Renal Insufficiency Cohort (CRIC) study[136] and the German Chronic Kidney Disease (GCKD) study—which will focus on kidney function and CKD progression.

Review criteria

A literature search was performed in PubMed using the search term "chronic kidney disease" AND "progression". Bibliographies and discussion sections of the identified articles were searched for additional relevant manuscripts. Papers discussing laboratory parameters found to predict the progression of chronic kidney disease were included when human studies were available that included a prospective observation period. No date restrictions were placed on the search.

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