Integrated genomics and metabolomics in nephrology

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ABSTRACT

Applying ‘omics’ approaches such as genome-wide association studies (GWAS) and metabolome analyses, genes and metabolites have been identified to be associated with renal pathophysiology. Meta-analyses of GWAS from large epidemiologic cohorts uncovered several novel loci linked with estimated glomerular filtration rate and chronic kidney disease (CKD). Sophisticated analytical technologies, including mass spectrometry and nuclear magnetic resonance spectroscopy, allow the analyses of up to 4000 targeted and non-targeted metabolites in plasma, serum and urine. Several uraemic toxins were found that were increased in CKD. Among them, arginine derivatives like asymmetric dimethylarginine or tryptophane metabolites have been identified as promising candidates to target mechanisms of kidney disease progression. This review aims to summarize recent findings in clinical kidney diseases research revealed by ‘omics’ approaches with a clear focus on recent genomics and metabolomics efforts.

Keywords: chronic kidney disease, genomics, metabolomics, nephrology

INTRODUCTION

Complex kidney diseases like chronic kidney disease (CKD), diabetic nephropathy (DN) or renal hypertension, involve a complex bundle of underlying pathophysiological mechanisms. Valuable insight has been acquired from candidate gene approaches to genome-wide association studies (GWAS); however, the underlying pathophysiological mechanisms do not only involve different genotypes but also changes in cellular, blood and urinary metabolites. Metabolomics complements other ‘omics’ data and as a downstream result of gene expression, changes in the metabolome are considered to reflect the activities of the cell at a functional level (Figure 1). Building on a substantive body of work over the past few years, we discuss herein the current knowledge of genomics and metabolomics in kidney diseases.

GENOME-WIDE ASSOCIATION STUDIES

Great progress has been made in mapping genomic variations of common disease at a genome-wide level. GWAS use genomic variations, termed single nucleotide polymorphisms (SNPs), to identify regions of the genome associated with the disease status or a clinical phenotype [1, 2].

GWAS: THEORETICAL ASPECTS

By design, GWAS provide an unbiased survey of the effects of common genetic variants. SNPs chosen for GWAS typically have a minor allele frequency (MAF) of ≥0.05 and are selected to ‘tag’ the most common haplotypes. However, the power of detection depends directly on the sample size of the study.
population, the MAF, strength of linkage disequilibrium (LD) between the SNP and the causal variants and the effect sizes of the alleles [3]. Typically, millions of statistical tests have to be performed simultaneously leading to the requirement for extreme significance levels [4]. To address the issue of multiple comparisons, Bonferroni correction is used [5] (Figure 2).

Imputation, using information about LD patterns revealed by the HapMap project [6] and the 1000G project [7] has become an essential statistical tool in GWAS. By using this information, it is possible to ‘supplement’ directly genotyped SNPs from commercial arrays with millions of ‘free’ genotypes spanning the human genome at relatively low cost of computation time and additional statistical tests, resulting in increased power to detect associations [8]. As larger sample sizes increase the number of identified loci [1], nowadays meta-analyses of GWAS results of many individual studies are conducted to increase the sample size and thus the statistical power. Large, international GWAS consortia have been founded to conduct standardized Meta-GWAS including up to 100,000 individuals for common disorders, such as the Global Urate Genetics Consortium [9], the CKDGen Consortium [10] or the CHARGE Consortium [11]. A detailed overview about the concept of GWAS has been described elsewhere and reviewed in refs. [1, 12, 13]. Standardized, reproducible, precise and quality-controlled measurements for

**FIGURE 1:** From genotype to phenotype: integrating genome, transcriptome, miRNA, proteome and metabolome to improve knowledge of renal diseases (patho)mechanisms.

**FIGURE 2:** GWAS variants for kidney disease and its risk factors to date summarized in a Circos plot. The circle represents the chromosomes; SNP locations and corresponding nearest genes are indicated by bars according to the respective kidney disease phenotypes. CDK, chronic kidney disease; eGFR-Crea, estimated glomerular filtration rate creatinine-based; eGFR-Cys, estimated glomerular filtration rate cystatin C-based; ESKD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; IgA-NP, IgA nephropathy; DN, diabetic nephropathy.
the phenotypic characterization are additional important key aspects that need to be ensured when performing GWAS [14].

**Chronic kidney disease**

Several GWAS have been conducted and have expanded the measure for genetic variants for CKD and the surrogate measure for renal function estimated glomerular filtration rate (eGFR). Köttgen et al. [9] conducted a GWAS in 19,877 European individuals and identified SNPs in several genomic regions among them the UMOD gene, encoding the glycoprotein uromodulin, which is exclusively expressed in the kidney. In a follow-up study, the same group proved that urinary uromodulin concentrations were associated with the UMOD SNP rs4293393, and elevated uromodulin concentrations increased the 10-year risk of CKD by over 70% [15]. It was suggested that rs4293393 might be one of the factors responsible for the association of altered uromodulin concentrations and incident CKD and could serve as a novel disease marker for diagnostic testing [15]. In addition to UMOD, this first CKD-GWAS identified several other loci including SHROOM3, GATM–SPATAS1L, STC1 and an intergenic loci between the cystatin C (CST3) and cystatin-9 (CST9) genes were found in association with eGFR based on creatinine or cystatin C [9, 16].

Further collaborative projects of the CKDGen consortium [10, 17] included additional population-based cohorts leading to a total of over 90,000 [3] and 130,000 individuals [17], respectively. The increased sample size extended the prior knowledge and identified additional 13 [3] and 6 [17] novel loci for kidney function. A zebrafish model was used to demonstrate the importance of these genes with respect to renal function. Those genes are involved in nephrogenesis (ALMS1, VEGFA and DACH1), podocyte function and glomerular filtration barrier formation (DAB2 and VEGFA), angiogenesis (VEGFA), solute transport (SLC7A9 and SLC34A1) and kidney metabolism (PRKAG2 and GATM). Many of these loci had previously been associated with kidney diseases and disorders of renal function [16]. Further newly identified loci were previously not known to be related to renal function (MPPED2, DDX1, CASP9, SLC47A1, CDK12, INO80 and ATXN2) [3, 17].

**Albuminuria**

The presence of albuminuria is a risk factor for progressive CKD and identification of genetic risk factors for albuminuria may alter strategies for early prevention of CKD progression [18]. A meta-analysis performed by the CKDGen and CARe consortia identified a missense variant in the CUBN gene for both the urinary albumin-to-creatinine ratio and microalbuminuria in Europeans and African-Americans [18]. Twenty-year follow-up association analyses showed a 42% greater risk of developing persistent microalbuminuria in individuals carrying the risk allele in a high-risk population of type 1 diabetes patients [18]. Additionally, the CUBN risk locus has been shown to associate with end-stage renal disease (ESRD) [19].

Cubilin, encoded by the CUBN gene, is predominantly expressed in the apical brush border of the renal proximal tubular cell [20] and plays a key role in the receptor-mediated endocytotic reabsorption of albumin and other low-molecular-weight proteins, in conjunction with megalin and amnionless. Dysfunction of the megalin–cubilin system has further been implicated in the pathogenesis of DN in both animal and human studies. Those findings indicate that levels of albuminuria in the general population are determined not only by glomerular filtration, but partly also by tubular reabsorption [16, 18].

**Diabetic nephropathy**

Diabetic nephropathy (DN) is a devastating complication of type 1 and type 2 diabetes. The first GWAS for DN was performed in a Japanese population using a low-density array [21, 22] indicated ELM01 (engulfment and cell motility 1 gene) as a candidate gene conferring DN susceptibility. This locus was replicated in several independent studies [23, 24] further establishing its role in disease susceptibility. ELM01 activity associates with increased expression of extracellular matrix proteins, leading to the expansion and thickening of the glomerular basement membrane [25], both are characteristics of DN.

Additional loci associated with DN such as risk variants near FMRD3 (FERM domain-containing protein 3), CARS (cysteinyl-tRNA synthetase) and ACACB (acyl-coenzyme A carboxylase) and the ERBB4 gene have been identified and validated [16, 26].

**IgA nephropathy**

Primary IgAN is the most common glomerulonephritis. So far, three IgAN GWAS identified several susceptibility loci [27–29] including the major histocompatibility locus, the HORMAD2 locus and the CFH gene cluster, providing first insights into the traits’ genetic architecture. Based on these GWAS, a genetic risk score was newly refined including seven identified loci, showing a strong association with IgAN risk.

**Metabolomics**

Metabolomics describes analytical approaches that aim to identify (and quantify) small molecules (metabolites <1.5 kDa) in a single experiment. Metabolomics approaches can be used either as targeted or non-targeted analyses. Targeted analyses are applied in hypothesis-testing studies and focus on the detection and quantification of dozens to hundreds of characterized and annotated metabolites. Non-targeted analyses are hypothesis-generating approaches that aim to comprehensively detect as many metabolites as possible without a priori knowledge [30]. Compared with other ‘omics’ technologies, there are fewer metabolites (3 × 105) than there are genes (2 × 106), transcripts (>106), proteins (>109) and post-translational modified proteins (>1010). Interpreting metabolomics is challenging due to sample handling and preparation, and application of several methodical platforms but also due to environmental factors such as circadian fluctuations or diet-dependent variability.
Currently, there is no single-instrument platform that can cover all metabolites; therefore, two main technologies, i.e. nuclear magnetic resonance spectroscopy (NMR)—and mass spectrometry (MS), are applied.

### Nuclear magnetic resonance spectroscopy

NMR spectroscopy is able to provide detailed molecule structure information that allows accurate structure elucidation. NMR-based metabolomics has the advantage that it is non-destructive and allows for absolute quantification without the use of stable-isotope labelled internal standards with a high analytical reproducibility; NMR signals correlate directly and linearly with compound abundance. NMR analysis requires neither elaborate nor time-consuming sample preparation (derivatization steps, for analyte separation). However, NMR equipment costs are extremely high. Although, sensitivity has continuously improved, MS-based techniques are much more sensitive, leading to a much lower detection limit than NMR approaches. A further drawback, especially for samples that are limited in their volume, is the relatively high amount of sample volume that is required for NMR analysis [30].

### Mass spectrometry

MS-based technologies are widely spread and routinely used in the pharmaceutical industry for drug metabolite identification and quantification and for therapeutic drug monitoring. Advantageously, MS-based technologies offer a higher analytical sensitivity than NMR and may therefore detect metabolites below the NMR detection limit. Due to accurate mass measurements, MS represents a powerful tool to analyse a larger number of metabolites. However, MS requires elaborate preparation steps including ionization and derivatization; both can cause metabolite losses. MS-based techniques can be used for both direct flow injection or coupling to a chromatography system in order to provide a greater specificity and to overcome MS-related problems like ion suppression or matrix effects. Mostly, chromatographic separation is achieved by gas chromatography (GC), capillary electrophoresis (CE) or liquid chromatography (LC) with LC approaches continuously evolving [e.g. ultra performance liquid chromatography (UPLC)]. In addition to the wide variety of separation techniques, there are several distinct analytical technologies for sample ionization [e.g. electrospray ionization or matrix-assisted laser desorption/ionization (MALDI)] and subsequent mass analyser for determining the mass-to-charge ratio (m/z) of the ions [e.g. time-of-flight (ToF), quadrupole-, ion trap-, Fourier transform (FT)-MS or combinations].

Metabolites and metabolic ratios are linked to changes in kidney function by three mechanisms: (i) metabolites that accumulate in tubular cells, blood or urine due to an impaired renal function. A well-known example is creatinine. (ii) Metabolites that reflect enzyme activity primarily expressed in kidney tissue. Examples are amino acids like N-acetylornithine [31]. (iii) Metabolites being uraemic toxins and contributing directly to the progression of diseases. Examples are indoxyl sulphate and asymmetric dimethylarginine (ADMA; see European Uremic Toxin Work Group EUTox, www.uremic-toxins.org). Besides the primary relation to kidney function, several metabolites reflect metabolic changes due to underlying diseases and secondary renal impairment. Alterations in energy metabolism in DN, i.e. impaired glucose uptake and enhanced β-oxidation are prominent examples. A summary of recent metabolomic investigations performed in different entities of kidney diseases is given in Table 1 and by Weiss and Kim [32].

### Chronic kidney disease

Changes in glucose, steroid hormone, purine, NO, tryptophan as well as in lipid metabolism have been implicated with the different stages of CKD [41, 42]. Several uraemic solutes represent interesting features. Alterations in the NO pathway [i.e. increase of ADMA and symmetric dimethylarginine (SDMA) and decrease of ornithine with increasing stages of CKD] were found in plasma by applying UPLC- and CE-MS [40, 41]. These alterations may reflect an impaired NO bioavailability which subsequently would promote atherosclerotic burden. Another interesting feature of metabolomic investigations in CKD is tryptophan depletion accompanied by an increase of several tryptophan metabolites, such as kynurenine, kynurenic acid and indoxyl sulphate [40, 42]. Tryptophan decay and increase in kynurenine and kynurenic acid may reflect a high indoleamine 2,3-dioxygenase (IDO) activity, which is an essential component of T-cell inactivation. Indoxyl sulphate is a tryptophan metabolite derived from gut bacteria that has been implicated in tubular cell dysfunction. Indoxyl sulphate down-regulates the renal-specific organic anion transporter (OAT) SLC04C1 [47]. Guanidinosuccinate, a known substrate for SLC04C1, and other carboxylate anions were found increased in CKD [40, 41]. Other OAT, i.e. OAT-1, are implicated in the clearance of indoxyl sulphate, kynurenine and xanthurenic acid, and might be therefore also pathophysiologically relevant in CKD [48].

### Diabetic nephropathy

Known metabolomic biomarkers of diabetes are sugar metabolites (i.e. 1,5-anhydroglucoitol), keton bodies (i.e. 3-hydroxybutyrate), free fatty acids and branched chain amino acids; all of them have been replicated in multiplatform metabolomic studies of diabetes [49, 50]. However, it is much more challenging to discriminate metabolites related to progression of kidney disease in diabetic patients. Distinct phospholipids, sphingolipids and sphingomyelins in serum and plasma have been identified in DN in type 1 and type 2 diabetic patients. An increase in acylcarnitines and a decrease of hippuric acid in urine are associated with early kidney damage, reflecting alterations in β-oxidation and uraemic toxin elimination, respectively [45]. Progression from micro-DN to macro-DN was accompanied by an increase of γ-butyrobetaine and decrease in azelaic acid (β-oxidation), as well as an increase in citrulline, SDMA and kynurenine [46]. IgAN and membranous
nephropathy (MN) have been characterized by perturbation in the Krebs cycle (i.e. cis-aconitate), increased protein degradation (i.e. tyrosine) as well as lipid metabolism [i.e. phosphatidylcholines (PC) and adipate, in IgAN and MN, respectively] [51, 52]. These phenotypes reflect similar alterations also found in experimental DN [53].

**Acute kidney injury**

According to the risk, injury and failure and loss and end-stage kidney disease criteria, acute kidney injury (AKI) is defined as a 2-fold increase of serum creatinine, a >50% decrease of GFR or a urine output <0.5 mL/kg/h within 12 h. The validity of this definition has been questioned and new criteria for diagnosis of AKI have been requested. Non-targeted UPLC-ToF has been applied to identify changes in serum metabolite profiles of AKI patients [33]. Besides increased creatinine levels and alterations of lipid metabolism (i.e. increase of acylcarnitines and decrease of lyso PCs) an activation of NO and oxidative stress pathways (i.e. ADMA and homocysteine) was observed. Furthermore, in AKI in children after cardiac surgery, a dopamine metabolite (i.e. homovanillic acid sulphate) was found increased in urine applying non-targeted LC-MS [36]. Experimental data provided evidence that alterations in lipid metabolism as well as nitrosative stress are a consequence of kidney ischaemia/reperfusion injury [54]. Other NMR-based studies identified bacterial metabolites to accumulate (i.e. trimethylamine-N-oxide; TMAO) or to be impaired (i.e. hippuric acid) in urine of patients with drug-induced AKI [34, 35]. In an experimental study of acute nephrotoxicity increased TMAO and dimethylamine (DMA) were associated with papillary dysfunction while suppression of hippuric acid and citric acid was related to proximal tubular damage [55]. Interestingly, similar metabolic patterns are also found in acute and chronic inflammatory kidney diseases like urinary tract infection [56] and GN [57, 58], respectively.

**Kidney transplantation**

Metabolomics in kidney transplantation have been applied to assess reperfusion injury and to predict allograft rejection. Using MALDI-FTMS, a fingerprint of unidentified urinary metabolites was applied to predict acute tubular injury and

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<th>Specimen</th>
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ADMA, asymmetric dimethylarginine; CE, capillary electrophoresis; GC, gas chromatography; LC, liquid chromatography; lyso-PC, lyso phosphatidylcholine; MOPEG, 3-methoxy-4-hydroxyphenylethene glycol sulphate; NMR, nuclear magnetic resonance spectroscopy; P, plasma; S, serum; SDMA, symmetric dimethylarginine; ToF, time-of-flight; TMAO, trimethylamine-N-oxide; U, urine.
necrosis as well as allograft rejection [59]. Major issues in transplantation are episodes of graft dysfunction and treatment optimization with immunosuppressant drugs. Bacterial metabolites like TMAO and DMA as well as metabolites of the Krebs cycle (i.e. citrate and succinate) were found elevated in the urine of patients after kidney transplantation [37, 38]. In another longitudinal study, NMR revealed increased bacterial and energy metabolites in serum before transplantation with a subsequent decline after kidney transplantation in recipients with normal graft function [39]. These alterations in kidney energy metabolism are substantiated by in vivo imaging studies and might be considered to identify patients with pathological changes after renal transplantation [60, 61].

**End-stage renal disease**

ESRD is the final stage of CKD. Likewise, similar metabolites have been found increased in plasma, including tryptophan metabolism, dimethylarginines, β-oxidation products and metabolites like TMAO, DMA and (p-hydroxy) hippuric acid [43, 44, 62]. Hippuric acid and p-hydroxy hippuric acid originate from gut bacterial degradation of non-absorbed phenylalanine and tyrosine, respectively, the latter has been implicated in the progression of ESRD by inhibiting tubular transmembrane Ca²⁺-transport [62]. Neurohormonal activation and impaired NO bioavailability are common features of CKD progression and renal failure, reflected by increased plasma concentrations of norepinephrine, dopamine and serotonin metabolites as well as increased ADMA/SDMA concentrations [43, 63]. Applying GC-/LC-ToF and NMR, several groups investigated adequacy of haemodialysis (HD) and peritoneal dialysis (PD) [64, 65]. In general, investigators found an increase of nucleosides and their metabolites [i.e. (hypo)xanthine, (1-methyl)inosine and guanosine], probably indicative of cellular hypoxia under HD [43, 65]. PD was characterized by an increase of glucose and other sugar metabolites, most likely attributed to the glucose content of the PD solution [64, 66].

While epidemiologic evidence for the link between kidney function (eGFR) and metabolites originates from cross-sectional studies [41, 67, 68], data from longitudinal cohorts are sparse. In two recent studies, spermidine, choline, citrulline and the ratio of distinct PCs and of kynurenine over tryptophane were identified as markers of incident CKD [69, 70]. The kynurenine over tryptophane ratio reflects increasedIDO activity possibly indicating a subclinical inflammatory process related to atherosclerosis [42, 71]. Another challenging task is the diagnosis and treatment of renal impairment. Worsened clinical outcome and disease progression might involve several decades of the patient’s lifespan. To adequately address this issue and optimize treatment reliable surrogates are needed. In the last years, several studies have focused on arginine derivatives as promising candidates to identify individuals at risk as well as to target treatment in kidney diseases.

**GWAS of metabolites**

Integrating ‘omics’ data sets has emerged as an approach to gain more functional information of the identified loci. As metabolites are suggested to play key roles in diseases, integration of genomics and metabolomics data (mGWAS) might provide novel biological insight and expand the current knowledge on the determinants of circulating metabolites.

Suhr et al. have recently published the first mGWAS using metabolites measured in human urine of the Study of Health in Pomerania (SHIP) [72]. Five loci were identified and replicated, i.e. AGXT2 (associated with 3-aminoisobutyrate), NAT2 (associated with formate/succinate ratio), WDR66 (associated with 2-hydroxysobutyrate), SLC7A9 (associated with lysine/valine ratio), SLC6A20 (associated with alanine/N,N-dimethylglycine ratio). The variance in metabolites explained by these loci ranged between 3 and 33%. Three of these loci were already described as risk loci for CKD: SLC7A9, NAT2 and SLC6A20, suggesting a plausible relationship between the metabolic traits, the encoded protein functions and disease [72]. A recent mGWAS by Rhee et al. [73] studied the human metabolome in the Framingham Heart Study and identified several novel loci associated with plasma metabolite levels, including the previously identified CKD loci. An association between the SLC7A9 locus (encoding a kidney amino acid transporter) and N⁵-monomethylarginine (NMMA), with the minor allele associated with a lower CKD risk and lower NMMA plasma levels. Interestingly, several study participants with normal kidney function at the time of metabolite profiling developed new-onset CKD in the following years, and higher plasma levels of NMMA were significantly associated with the risk of developing future CKD [69, 73]. These results highlight plasma NMMA as a potential intermediary between common variant at the SLC7A9 locus and renal disease and suggest NMMA as a biomarker of CKD risk [69]. In addition to NMMA, the study of Rhee et al. identified further kidney disease-related loci that showed associations with metabolites: CPS1 and glycine and creatinine, GCKR variants and alanine, lactate, hydroxybutyrate and several triacylglycerols. In summary, results of these mGWAS demonstrate that genetic variability causes differences in the individual metabolic content, leading to novel approaches such as individualized medicine.

**NEXT STEPS: WHAT DOES THE FUTURE HOLD?**

Valuable insights into the molecular genetics of kidney diseases were acquired and major advances in our knowledge of the genetic and metabolic structures have been made. Yet, ≤2% of the estimated heritability has been explained [1]. Unravelling the complexity of kidney diseases will require a detailed understanding at the molecular and cellular level. With rapid advances in next-generation technologies, whole-exome and even whole-genome sequencing is feasible and provides a comprehensive map of novel, rare variants in the human genome. In addition, there is a need for novel, reliable biomarker for early diagnosis and prediction of kidney disease progression and treatment decisions. The application of metabolomics emerges as a promising approach for biomarker discovery. By continuously evolving technological and analytical tools, metabolic profiling does not only focus on a single marker but rather on a panel of
metabolites, providing a more detailed description of the biological and pathological processes and thus, for the identification of novel metabolite biomarker. The systematic integration of the multidimensional datasets of ‘omics’ and the translation into biologically meaningful functions evolves as the next challenge for the future. Interactions between proteins, metabolites, transcripts, regulatory RNAs and DNA will provide a more comprehensive picture. Modern systems biology networks will help to understand the function of the whole organism, using information from a wide variety of disciplines and novel effective computational models (e.g. KEGG Pathways, Human Metabolite Database, LIPID MAPS Lipidomics Gateway, Ingenuity Pathway Analysis). Recently, Pesce et al. [74] have extensively reviewed a holistic approach by integrating experimental data from ‘omics’ technologies with mathematical models and computational tools in nephrology. On the basis of this, approaches for personalized medicine in kidney diseases and subsequent individualized treatment options can be implemented.

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CONFLICT OF INTEREST STATEMENT

None declared.

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