How can genetics and epigenetics help the nephrologist improve the diagnosis and treatment of chronic kidney disease patients?

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ABSTRACT

Discovery of novel improved tools for diagnosis, prevention and therapy of chronic kidney disease (CKD) is an important task for the nephrology community and it is likely that scientific breakthroughs, to a large extent, will be based on genomics. The rapid growth of the number of genome-wide association studies, major advances in DNA sequencing and omics profiling, and accelerating biomedical research efforts in this area have greatly expanded the knowledge base needed for applied genomics. However, translating and implementing genotype-phenotype data into gene-based medicine in CKD populations is still in an early phase and will require continuous research efforts with integrated approaches and intensified investigations that focus on the biological pathways, which causatively link a genetic variant with the disease phenotype. In this article, we review some current strategies to unravel these translational gaps as well as prospects for the implementation of genetic and epigenetic methods into novel clinical practice.

Keywords: chronic kidney disease, clinical utilization, epigenetics, genetics, GWAS

INTRODUCTION

Patients suffering from chronic kidney disease (CKD) are at considerable risk of premature death [1, 2]. Although important therapeutic developments have been made, most current treatments, such as dialysis, are rather unspecific. Therefore, it remains a fundamentally important task for the renal research community to seek new ways and strategies to modify and improve disease interventions to achieve more specific, efficient and individualized therapies. Gaining increased knowledge of the underlying molecular mechanisms of CKD and its complications is a crucial step in this process.

The exponential increase in genome-wide association studies (GWASs) has vastly improved the understanding of the genetic make-up of CKD and revealed previously unappreciated disease pathways and mechanisms, potentially representing targets for therapeutic interventions [3]. The clinical usefulness of the genetic information may, however, only be realized once functional links between genotypes and phenotypes have been validated. For the majority of the hitherto reported CKD-associated loci, the effects on disease pathogenesis are still largely unknown. Thus, interpreting GWAS findings for translational research is a main challenge ahead. Although this daunting task is both laborious and complicated, several approaches are being established. These include studies that integrate GWAS data with information generated from human tissue-specific expression, systems biology and animal experiments to reveal variants potentially affecting e.g. gene regulation or protein function [4]. In a previous review in this series [5] we described how novel insights from genetic and epigenetic studies are helping us to better understand the complex uraemic phenotype. In this second review article, we discuss how genetics and epigenetics can help the nephrologist to improve the diagnosis and treatment of CKD patients (Figure 1). In the following we briefly discuss current approaches to unravel mechanistic links between genetic variants and disease and how novel insights into biologically relevant pathways could potentially translate into clinically meaningful strategies, and finally the exciting but tortuous road towards utilization of this new knowledge into clinical practice.
FIGURE 1: The complex CKD phenotype is determined by interacting environmental, genetic and epigenetic factors. These factors are not completely understood but, during the last decade, whole-genome gene chips and improved DNA sequencing techniques have generated a burst of data from genetic and epigenetic association studies (I), which have helped nephrologists to elucidate novel pathogenic pathways. The subsequent translation of these genotype/epigenotype–phenotype data into relevant biological mechanisms entails multi-discipline approaches, including transcriptomics, metabolomics, proteomics and functional *in vivo* studies (II). Information on genetic/epigenetic risk profiles and links to disease phenotype and renal pathophysiology may be utilized to develop new genetic/epigenetic-based tools for clinical care of CKD patients, e.g. diagnostics, risk prediction and personalized interventions and therapeutics (III).

TRANSLATING GENETIC ASSOCIATIONS INTO BIOLOGICAL INSIGHT

Strategies to resolve the translational gap between disease-associated variants and phenotypic end points include studies on the downstream effects of genetic variations. A promising approach is to study the intermediary molecules that act in the reaction chain between genotype and phenotype, e.g. RNA, proteins and metabolites, to identify the functional context (Figure 1). Further elucidation of functional mechanisms may be conducted via systems biology approaches, regulatory assessments and model organisms.

**Integrating genetic variation and gene expression data**

Variation in human gene expression, i.e. the transcription of genes into RNA molecules, has been shown to be highly relevant for studies aiming at providing functional support for disease-associated genetic variants. Dysregulated gene expression may directly contribute to complex diseases and is also often genetically regulated [6]. In addition, since ~90% of the currently identified genetic loci are localized outside protein-coding sequences, it is likely that these play a role in gene regulation and gene expression rather than directly impacting the protein product [7]. In this way, gene expression represents an essential and quantifiable intermediary link between genotype and phenotype. Single nucleotide polymorphisms (SNPs) that associate with gene expression may contribute to diseases that result from disruption of gene regulation homeostasis. This may include transcriptional mechanisms, such as aberrant gene splicing, transcription factor binding, enhancer elements or messenger RNA degradation, [8]. Human polymorphisms affecting microRNA (miRNA) target sites [9], DNA methylation sites [10] and histone modifications [11] should be of particular interest for studies linking GWAS data and gene expression profiles. MirSNP database [9] and Human Epigenome Project and the epigenomics initiative of the National Institutes of Health (http://commonfund.nih.gov/epigenomics/epigeneticmechanisms.aspx) provide helpful tools for identifying putative genetic variants that affect epigenetic regulation of gene expression. In addition, assessing associations between different gene transcripts may reveal potential co-regulatory gene networks [4]. Combining such networks with data on genetic variants and pathway analysis information renders it possible to identify multiple gene-regulatory effects of disease-associated variants.

Previous studies on associations between genotype and quantitative levels of expression, so-called expression quantitative trait loci (eQTLs) analysis [8, 12], have successfully uncovered genetic variants associated with gene expression in diseases, such as obesity [6] and cardiovascular disease (CVD) [13]. The increasing number of large-scale genetic and gene expression studies is expected to facilitate integrated studies on genetic and gene expression data also in CKD populations. One important restraint, however, is the limited availability of adequate target tissues, and multinational joint efforts are therefore being initiated to overcome this obstacle. For instance, the European Renal cDNA bank [14–16] is collecting gene expression data from renal biopsies, and the CKDGen [17] provides phenotypes and genotypes in a large European collaboration that aims to identify links between gene variants that influence expression and glomerular filtration rate traits. So far, eQTL studies on renal traits have contributed to the discovery of a genotype association with kidney aging in humans [18] and identification of an association between a novel loci (from a European GWAS on kidney function) and expression of CASP9, which encodes a protein involved in cell apoptosis, necrosis and inflammation [19]. Other initiatives, such as the Genotype-Tissue Expression (GTEx) project, pursued by the GTEx consortium, strives to build a resource database for
studies on the relationship between genetic variation and gene expression in a more comprehensive selection of human tissues [20]. Thus, the prospects for identifying gene-regulatory functions and higher order networks for disease-associated SNPs are greatly improved by current analytical approaches that merge genome-wide assays of gene expression and genetic variation. In addition, combining such integrated databases with information on other molecular phenotypic data, e.g. epigenomic information, may further enhance the knowledge about the biological impact of a gene variant.

**Functional annotations and systems biology**

The purpose of extracting detailed annotations of the genome is to facilitate mapping of genetic variants that associate with gene-regulatory regions and expression, and subsequently, assess plausible links to human disease. A milestone in this area was reached in 2012, when the Encyclopedia of DNA Elements (ENCODE) project published an extensive report on regions of transcription, transcription factor association, chromatin structure and histone modification in the human genome [21]. Their updated database, the GENCODE 7 (gencodegenes.org) [22], was recently launched and is available for genetic researchers.

Further important developments are being made within the field of systems biology to identify functional links between genotype and phenotype. Systems biology tools rely on a multidisciplinary approach, in which whole-genome data are merged with quantitative data from transcriptomics, metabolomics and proteomics, to establish and validate biological models, e.g. metabolic or cell signalling networks that signify a cell, tissue or organism. By obtaining a more holistic view on the complex interactions within different biological systems, functional genomics information can be derived. An example of a novel functional genomics approach that combined regulatory SNP prediction, transcriptional promoter modelling and pathway analysis was recently reported by Martini et al. [23].

The authors successfully applied the method to assess the functional context of a non-coding gene variant (rs1888747) found in a genetic region adjacent to the FRMD3 promoter and that was strongly associated with diabetic nephropathy (DN) [23]. Similarly, via integrative analyses of genotype data, expression data and genome-wide binding profiles of a well-recognized transcription factor, NFκB, a recent study was able to reveal associations between NFκB binding and disease-associated variants in a genotype and allele-specific manner [24]. Another integrated experimental and computational system approach was utilized to identify a protein kinase, homeo-domain interacting protein kinase 2 (HIPK2), as a critical mediator of gene regulation changes that contribute to the pathogenesis of human kidney fibrosis [25]. Assigning putative functions to disease-associated variants and gaining improved understanding of the functional elements of the genome in the biological context are clearly important as such data may be utilized to prioritize SNPs for further experimental validation in animal models.

**Animal models for studying biological function**

Since animal models enable unique means to interfere with biological processes, such systems are well fitted to establish functional validation of genetic associations. The zebrafish is considered a particularly suitable model organism for studies on genetics and kidney biology: gene function and basic cellular processes are highly conserved between zebrafish and mammals; kidney development is easily visualized in the embryos and several analytic tools for intervening with cell biology and genetics in the zebrafish are available [26].

Thus, functional evaluation of novel GWAS-derived genes, associated with kidney function in Europeans [19] and African Americans [27], was recently conducted in zebrafish in two separate studies. The European follow-up study performed knockdown of mpped2 and casp9 through Morpholino antisense oligos in zebrafish embryos, yielding podocyte and tubular abnormalities with altered dextran clearance [19]. Similarly, the biological functions of kcnq1, dok6 and fndc1, reported from the Candidate-gene Association Resource Renal Consortium, were tested with Morpholino knockdown in zebrafish. Whereas depletion of dok6 and fndc1 did not have any effect, kcnq1 knockdown resulted in abnormal kidney development and filtration capacity, supporting a role for kcnq1 in glomerular development [27]. These studies provide evidence for a causative link between disease-associated variants, genes and novel molecular mechanisms that seem to be important for renal function, e.g. podocyte, tubular and glomerular development, in a true biological context. In addition, these pioneering studies illustrate that zebrafish may serve as a useful in vivo model to conduct functional genetic studies on CKD-associated genes generated from GWASs. Continuous investigations in the zebrafish as well as in other animal models are, however, needed to further assess functional implications for the multitude of GWAS-identified genes. For instance, the hibernating bear (Ursidae) [28] as well as hummingbirds, diving seals and vampire bats [29] have been proposed as highly interesting and novel animal models for kidney research. Translational studies including genetic information, cell biology and animal and human data should have great potential to solve pathogenic mechanisms acting in renal disease, and may lead to novel therapeutic interventions for CKD patients.

**Clinical implications of genetic knowledge in CKD diagnosis and care**

Although the practice of using genetically informed therapeutic choices is only in its infancy, genetic testing of steroid-resistant nephrotic syndrome [30] and haemolytic uraemic syndrome/thrombotic thrombocytopenic purpura patients [31] for diagnostic purposes is already being suggested. This field is likely to develop within a near future and here we discuss some highly interesting reports where genetic information is conquering diagnostic and therapeutic areas within nephrology.

**Genetic dissection of complex kidney disease phenotypes**

In the era of personalized medicine, it is vital to be able to precisely diagnose and evaluate homogeneous subgroups of CKD patients with different underlying renal diseases as this may help in targeting adequate treatments and improve
prognostic estimates. The clinical value of using genetic information for phenotypic stratification was recently illustrated in a case study of a patient with long-standing Type-2 diabetes, who was examined both by myosin, heavy chain 9, non-muscle (MYH9) E1 genotyping and renal histology [32]. Following the observation that the examined patient was homozygous for all MYH9 E1 risk variants that strongly associate with non-diabetic end-stage renal disease, ESRD [e.g. focal segmental glomerulosclerosis (FSGS)] in African Americans [33–35], the researchers scrutinized the histological specimen and detected lesions typical not only for DN but also for FSGS. Because DN and FSGS patients do not respond to the same therapies, the clinical impact of identifying a mixed diagnosis variant is great. For instance, potential glucose-lowering therapies will most likely have a more beneficial effect on disease progression in those patients with DN without coexisting FSGS and who do not carry MYH9 risk alleles.

Further multidisciplinary approaches with potential to advance medical management of complex renal diseases are under way. For example, the North American multicentre collaborative consortium NEPTUNE (Nephrotic Syndrome Study Network) is pursuing a large cohort study in which clinical, histopathological, gene expression and genetic data are integrated [36]. The study set-up entails clinical and molecular phenotyping at the time of the indicated renal biopsy and with this information the consortium aims to identify clinical, histological and genomic disease predictors. In the optimal scenario, genetic dissection of the cause of kidney disease will provide clinicians with new diagnostic routines, without the need of obtaining renal biopsies.

**Personalized therapy using ethnic-difference markers**

Previous data emphasize the importance of considering population ancestry disparities in kidney disease risk and treatment response. The role of essential hypertension in the etiopathogenesis of ESRD, and in particular in non-diabetic hypertension-associated ESRD, has been a controversial enigma since African Americans with hypertension-attributed CKD, who are successfully treated with blood pressure lowering medication, still progress to ESRD more rapidly than CKD patients of European ancestry. However, mapping by admixture linkage disequilibrium [37], using ethnic-difference markers, identified a region on chromosome 22q that was associated with renal disease in African Americans [34] and further studies of the MYH9 and apolipoprotein L1 (APOL1) genes located in this segment, have revealed that while MYH9 risk alleles are associated with non-diabetic CKD and DN in European Americans [38, 39], APOL1 risk alleles are strongly associated with FSGS and hypertension-attributed ESRD in Americans of African descent. To further explore the impact of MYH9 and APOL1 variants in hypertension-attributed CKD, Lipkowitz et al. [40] performed a case–control study including patients with clinically diagnosed hypertensive nephropathy from the African American Study of Kidney Disease and Hypertension (AASK) and African American healthy controls with mild-to-moderate hypertension. The analysis showed that APOL1 risk alleles G1 and G2, and to a lesser extent MYH9, are associated with CKD attributed to essential hypertension in non-diabetic AASK participants. Interestingly, APOL1 risk alleles predicted the progression rate of the disease irrespective of medication class and blood pressure treatment arm, suggesting that the poor kidney-protective response to medication is also associated with APOL1 risk variants. Very recent prospective data from the AASK and the Chronic Renal Insufficiency Cohort support the observation that African American patients with APOL1 high-risk alleles are subjected to an increased risk of disease progression, despite a well-controlled blood pressure and regardless of diabetes status, and suggest that the APOL1 genotypes do not modify the effects of proteinuria and the treatment regimens tested [41]. Considering that >50% of African Americans carry at least one risk allele [42] and hence do not attain kidney protection despite intensive treatment of blood pressure, there is an urgent need to understand the biology of APOL1-associated renal disease to identify new targets for development of effective therapeutic interventions. The APOL1 genotype may also have implications for renal allograft survival, since it has been shown that kidneys donated from deceased African Americans carrying two risk alleles fail more rapidly after transplantation than those from donors with no or one risk variant [43]. Therefore, APOL1 screening may improve the donor selection process and maximize long-term renal allograft survival.

**Risk prediction with genetic risk scores**

DNA variants are believed to become better biomarkers for early prediction than for example later occurring changes in protein levels, and GWAS-based genetic risk scores are being tested also in the context of CKD. For instance, a genetic risk score for CKD stage 3 prediction was recently generated based upon novel data, reported by the CKDGen consortium [44], on risk loci associated with kidney function and CKD [45]. In total, a panel of 16 SNPs were selected to construct the genetic score. The statistical analyses showed that whereas each risk allele added another 6% to the relative risk of CKD 3 after adjusting for age and sex, the genetic risk score did not perform better than standard clinical risk factors to predict new cases of CKD stage 3. Clearly, as this research area is in a very early phase and as genetic knowledge will increase and more CKD risk variants will be uncovered, it will likely be possible to generate improved risk algorithms with better discriminatory power in the future.

In another setting, genetic scores were used in an attempt to clarify the causal relevance of low-density (LDL-C) and high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels in the development of carotid atherosclerosis and the aptness of carotid intima–media thickness (CIMT) as a surrogate biomarker in clinical trials of therapies targeting these lipids [46]. The causality between high LDL-C levels and increased risk of coronary heart disease (CHD) has been established in randomized clinical trials and LDL-C lowering treatments such as statins reduce CHD risk in proportion to LDL-C reduction [47]. However, randomized trials have not been able to confirm the positive effects of medications increasing HDL-C or reducing TG levels [48]. Based on SNP data from a gene-centric array (Cardiochip scores) and a genome-wide
association meta-analysis (Global Lipids Genetic Consortium scores), genetic scores specific for predicting LDL-C, HDL-C and TG concentrations were generated and subsequently used in a Mendelian randomization approach to determine the relationship between the lipid fractions and common CIMT. The analyses were able to confirm the causal association between LDL-C and CIMT only, and hence the authors concluded that CIMT may be less appropriate as an end-point surrogate measure for HDL-C and TGs [46].

Recent efforts have also been made to create personal omics (transcriptomic, metabolomic and proteomic) risk profiling to gain improved understanding of the individual’s genetic make-up and disease risks. In 2012, Chen et al. [49] coupled longitudinal omics profiling with genome sequencing and were able to decipher dynamic intra-individual changes during healthy and diseased states, suggesting that such detailed molecular and physiological information may be of significance for personalized health monitoring and medicine. However, longitudinal patient monitoring for tracking disease onset and progression puts high demands on the capability of storing data and present ethical dilemmas regarding data security and risk of genetic discrimination that need to be solved.

Genotype-guided lifestyle interventions and therapeutic targets

Relationships between genetic make-up and how well the body will respond to different therapeutic options, e.g. dietary interventions and targeted therapeutic efforts, are of great clinical interest and studies addressing this issue are receiving increased attention. For instance, weight loss interventions are commonly advocated to decrease blood pressure in hypertensive patients, but not all patients achieve a desirable response. Recent genetic association data showing the association between genetic variants and weight sensitivity suggest that prior evaluation of genotype may be one way to identify individuals who may benefit more from weight loss, when compared with other types of lifestyle interventions [50]. In addition, information on these gene variants may give an indication of the expected effect on blood pressure in relation to a given amount of weight loss, which should be of importance in hypertensive CKD patients.

Previous studies also implicate associations between salt sensitivity, genotype and response to angiotensin-converting enzyme (ACE) inhibitors, hence recommending reduced dietary sodium intake to achieve better treatment effects in CKD individuals carrying the ACE deletion/deletion (D/D) genotype [51, 52]. Moreover, another deletion/deletion variant in the glutathione S-transferase M1 gene, involved in oxidative damage, infers a greater susceptibility to oxidative stress in HD patients, thus proposing the implementation of more potent antioxidant therapies in individuals with risk genotypes [53–55].

Considering the potential importance of lifestyle, it is of increasing importance to exploit the novel and emerging technologies for epigenome-wide analyses, since it is likely that new mechanisms will be revealed for the interactions between genotype and environmental/lifestyle influences that may explain individual disease vulnerability and resilience.

Pharmacogenomics

The need to evaluate the efficacy and responsiveness to a given therapy and to relate the results to genetic background in order to optimize the beneficial effects and minimize adverse drug reactions is becoming increasingly recognized in many clinical scenarios. Despite promising developments within oncology and CVD research [56] and although several efforts have been made to study gene–drug interactions in the field of nephrology, results are often discordant between different studies and populations, mainly because of methodological limitations due to inappropriate study design and difficulties in defining accurate phenotypic end points [57]. We discuss two important therapeutic areas in which drug efficacy could benefit from implementing genetic background information of patients.

Anti-hypertensive drugs in CKD. Hypertension is a major and independent risk factor for CKD progression, which in concert with proteinuria and albuminuria leads to increased cardiovascular morbidity and mortality [58, 59]. Hyperactivity of the renin–angiotensin system (RAS) is associated with renal damage progression and consequently blockade of the RAS is recommended as first choice treatment of hypertension in early-CKD patients [60]. A vast number of drugs targeting RAS have been developed, but there is a high inter-individual variability in pharmacological response and renoprotection and despite thorough medication, either by single or combined RAS drugs, many patients progress to ESRD or die from cardiovascular complications. Hence, the genetic characteristics of the RAS components are being extensively investigated and have already yielded evidence supporting association between RAS variants and pharmacogenetic responses. The most commonly used categories of RAS blockers target the angiotensin I-converting enzyme (ACE inhibitors) and the angiotensin II-receptor (AT1 blockers, ARBs) and polymorphisms in the respective genes ACE and AGTR1 as well as in the angiotensinogen (AGT) gene have been associated with both clinical phenotypes and differences in drug response. It has been shown that compensatory mechanisms may increase aldosterone levels and contribute to the development and progression of DN in patients on chronic treatment with RAS blockers [61]. The phenomenon occurs in as many as half of the treated patients, suggesting that this long-term inter-individual variability should be a focus in future pharmacogenetic studies. The ACE insertion/deletion (I/D) polymorphism in intron 16 has been the most frequently analysed when studying the genetic impact on RAS blocker efficiency. In a prospective randomized, clinical study it was demonstrated that, compared with placebo, ACE inhibition therapy reduced albuminuria with 51.3, 14.8 or 7.7% in patients carrying the I/I, I/D or D/D genotypes, respectively [62]. In Type 1 DM patients with DN, the I allele was associated with improved outcome following medication with ACE inhibitors, whereas results were inconclusive in patients with Type 2 DM. A multicentre clinical trial studying ARB treatment (losartan) in Type 2 DM patients showed that ACE D allele carriers had an unfavourable renal prognosis, which could be mitigated and even improved by losartan [63]. In a meta-analysis of randomized clinical trials of both ACE inhibitors and ARBs, including diabetic and non-
genes in the RAS pathway, such as \textit{REN} which may in increased oxidative stress, are thought to affect the epigenome, \textit{BDKRB2} (IL-6) levels require elevated ESA supplementation [68]. In level. For instance, ESRD patients with high interleukin 6 the ESA dose required to maintain the desired haemoglobin treatment, but there is a substantial inter-individual variability of treated by erythropoiesis-stimulating agent (ESA) supplemen-
tation, but there is a substantial inter-individual variability of the ESA dose required to maintain the desired haemoglobin level. For instance, ESRD patients with high interleukin 6 (IL-6) levels require elevated ESA supplementation [68]. In this regard, information that a genetic variant in the IL-6 gene, the IL6–174 G allele, is associated with both increased IL-6 levels and higher erythropoiesis-stimulating protein (ESP) dose in HD patients [69] suggests that it may be clinically relevant to include IL-6 genotyping in strategies that aim to develop individualized ESP dose recommendations.

\textbf{Anti-anaemic drugs in CKD.} Anaemia, one of the most frequent and expensive complications in CKD patients (USRDS 2005) is a result of reduced erythropoietin production and impaired haematoipoiesis, which is worsened by iron deficiency, lack of vitamins (i.e. folate and vitamin B12, essential for methyl donor production) and chronic systemic inflammation commonly seen in these patients and which further decrease erythropoietin effectiveness [68]. Today, most patients are treated by erythropoiesis-stimulating agent (ESA) supplemen-
tation, but there is a substantial inter-individual variability of the ESA dose required to maintain the desired haemoglobin level. For instance, ESRD patients with high interleukin 6 (IL-6) levels require elevated ESA supplementation [68]. In this regard, information that a genetic variant in the IL-6 gene, the IL6–174 G allele, is associated with both increased IL-6 levels and higher erythropoiesis-stimulating protein (ESP) dose in HD patients [69] suggests that it may be clinically relevant to include IL-6 genotyping in strategies that aim to develop individualized ESP dose recommendations.

\textbf{Targeting pathophysiological epigenetic changes in CKD}

Many unknowns regarding gene–environment interaction and epigenetic modifications hamper translation of genetic information into clinical applications. Studies on the epigenome may help to decipher molecular mechanisms and define novel means to interfere with CKD-related pathogenic processes. In contrast to genetic alterations, epigenetic modifications are reversible, susceptible to environmental cues, and display developmental and temporal variability [70]. Due to this dynamic nature, drugs directed towards epigenetic modifications, restrictions regarding adverse environmental exposure and dietary intake as well as other lifestyle interventions in the management of the epigenetic aspects of disease are likely to have beneficial effects. Some successful examples on epigenetic drugs are already being reported, primarily in cancer treatment [71].

Several pathophysiological features of CKD, including hyperhomocysteinaemia, low-grade chronic inflammation and increased oxidative stress, are thought to affect the epigenome, which may influence disease development [70]. As discussed in a previous article from our group [5], characterization of the CKD-specific epigenome is ongoing and may point to new therapeutic targets in CKD. For example, in an interventional study, administration of folate in ESRD patients with hyperhomocysteinaemia was shown to modulate DNA methylation status, suggesting that hyperhomocysteinaemia-induced DNA hypomethylation could be reversed by folate [72]. Additionally, pioneering genome-wide searches of epigenetic alterations in CKD have revealed several differentially methylated candidate genes associated with CVD and with immune/infection diseases [73] as well as epigenetic differences between diabetic patients with or without ESRD [74]. Since the differentially methylated genes appear to be involved in proatherogenic processes [73], pathways associated with DN and dialysis treatment [74], these DNA markers may serve as inter-individual predictive biomarkers of disease susceptibility, progression and treatment. Combined DNA methylation and mechanistic studies made in rodents further show that hypermethylation of the renoprotective gene \textit{KLOTHO} (a key regulator of phosphate balance) downregulates its gene expression and is a key event linking uraemic toxins to CKD progression, thus proposing \textit{KLOTHO} as a potential epigenetic drug target to suppress uraemic toxin-induced CKD progression [75].

Dysregulation of histone modifications has been highlighted in a wide range of renal diseases and complications, including renal injury, fibrosis, congenital anomalies of the kidney, renal hypoxia, diabetic renal complications and inflammation, and emerging discoveries are expected to open up new avenues for therapeutic targets in kidney disease [76]. For instance, findings from diabetic db/db mice treated with angiotensin II type 1 receptor (AT1R)-blocker losartan show that losartan not only ameliorates DN phenotype but also reverses histone modifications H3 lysine-9/14 acetylation in renal glomeruli [77], suggesting that improved treatment effects may be achieved with drugs that are directed both towards epigenetic regulators and traditional drug targets. In addition, the increasing number of miRNAs that are now being reported to play a role in human and animal models of CKD represents putative therapeutic targets in kidney disease [78]. Indeed, inhibition of miRNA activity with small molecule inhibitors such as antagonists, i.e. oligonucleotides that block miRNA-mediated silencing of miRNAs, is already being used as an epigenetic therapy for various cancers [79]. Moreover, the use of miRNAs for diagnostic purposes is highly promising: miRNAs are reported as being sensitive and stable biomarkers and can be measured in a plethora of body tissues, including easy accessible body fluids such as blood and urine [80].

Advances in affordable high-throughput methods to quantify epigenetic modifications and increased knowledge about epigenetic regulatory mechanisms will no doubt eventually serve to improve our methods for clinical diagnostics and therapeutic interventions in CKD. However, in contrast to research areas such as cancer, there is still a scarcity of epigenetic studies in renal disease, and therefore our understanding of epigenetic processes in the context of this disease remains limited. Furthermore, whereas studies on epigenetics will most likely be crucial for capturing dynamic aspects of initiation and progression of CKD and how the manifestations, intensity and duration of CKD are influenced by the combined impact of environmental changes and genetic predisposition, the dynamic nature of epigenetic state as such makes it a problematic moving target. In general, an important obstacle for
studies on epigenetics is that epigenetic profiles are time and space dependent and therefore likely to differ markedly, not only over time (diurnal, seasonal and developmental variations) but also between different locations (cell, tissues and organs) and between different nutritional (dietary intake and nutritional status) and environmental contexts (e.g. presence of conditions such as diabetes, uraemia, inflammation). Epigenetic studies in CKD are therefore highly challenging. On the other hand, several of these factors (e.g. amount and type of dietary intake, degree of uraemia as determined by dialysis) can be modified in a controlled manner. Thus, the complex situation in CKD may provide unique opportunities for scientific advances. Nonetheless, a substantial amount of thorough additional work is needed before new knowledge based on epigenetic regulatory mechanisms may become implemented in clinical practice.

SUMMARY AND CONCLUSIONS

It has become clear that the achieved advances in clinical care and dialysis techniques will not be sufficient to halt the increasing prevalence of CKD. Rather, this global health problem needs to be addressed in terms of fundamental disease understanding and identification of new risk factors as well as diagnostic, therapeutic and preventive targets. The cumulative data on disease-associated genetic variants has contributed to illuminate previously unrecognized pathophysiological pathways and opened up novel translational research. Efforts are now needed to confirm the functional links between genetic risk loci, interpreted by specific epigenetic mechanisms that lead to disease phenotype and renal pathophysiology. This is, indeed, a complex mission, which will require researchers to adopt a holistic approach, ranging from medical medicine to large epidemiological studies, with collaborations between several systems biology disciplines such as genomics, epigenomics, transcriptomics, metabolomics, proteomics and bioinformatics. Only when the translational gap is overbridged will we be able to fully comprehend the potential clinical utility of the genetic knowledge. Nevertheless, emerging data uphold great promises to implement genetic information for developing new tools to identify patients at risk, dissecting complex phenotypes, personalize therapies and predict variability in drug response as well as to guide the selection of preventive measures and lifestyle interventions in CKD populations. As cost and speed of high throughput genetic analyses have improved greatly, medical genomic and epigenomic sequencing are becoming increasingly feasible and a wealth of data sets integrating genomic and phenotypic health information is already available to facilitate clinical use of genomics and potentially also epigenetics.

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CONFLICT OF INTEREST STATEMENT

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