The rising number of older individuals in national populations has led to an increase in age-related diseases such as chronic kidney disease (CKD) (126, 127, 129). CKD is defined as a progressive loss of renal function, measured by a decline in glomerular filtration rate (GFR), which is typically associated with irreversible pathological changes within the kidney. Common etiologies for CKD include diabetes, hypertension, glomerulonephritis, polycystic kidney disease, and chronic pyelonephritis (28, 129, 130). CKD is normally classified into distinct clinical stages stratified on the basis of estimated GFR (eGFR) (58–60). eGFR measurements are derived from formulae based on the individual’s serum creatinine, age, sex, and ethnicity. Stage 1 CKD (eGFR > 90 ml·min⁻¹·1.73 m⁻²) and stage 2 CKD (eGFR = 60–89 ml·min⁻¹·1.73 m⁻²) are recognized in persons with other evidence of kidney disease, such as proteinuria or multiple renal cysts. For most clinical and genetic epidemiological studies, CKD has been defined as eGFR < 60 ml·min⁻¹·1.73 m⁻², irrespective of the presence or absence of kidney damage, and this includes stage 3 CKD (eGFR = 30–59 ml·min⁻¹·1.73 m⁻²), stage 4 CKD (eGFR = 15–29 ml·min⁻¹·1.73 m⁻²), and stage 5 CKD (eGFR < 15 ml·min⁻¹·1.73 m⁻² or persons on chronic dialysis) (59). Stage 5 CKD is also known as end-stage renal disease (ESRD). Although GFR is a quantitative trait, it has proved convenient for research purposes to define renal clinical phenotypes based on eGFR measurements, i.e., CKD (eGFR < 60 ml·min⁻¹·1.73 m⁻²) and ESRD (eGFR < 15 ml·min⁻¹·1.73 m⁻²).

Individuals with progressive CKD have an increased risk of cardiovascular events, hospitalization, and death (29). In addition, persons with CKD may have progressive loss of kidney function and eventually develop ESRD requiring renal replacement therapies (chronic dialysis and/or renal transplantation). The worldwide prevalence of individuals with ESRD is steadily increasing (21, 71, 112). In keeping with most common multifactorial diseases, CKD has a complex etiology involving both an inherited predisposition and exposure to environmental factors. Inherited risk factors for CKD have typically been considered to be manifest as genetic variants in the DNA sequence; however, more recently, epigenetic modifications of the genome have been identified that impact on kidney biology and the risk of kidney failure (Fig. 1). Epigenetic features may be stably inherited or dynamically change, providing a critical link between an individual’s genome (DNA) and their environment. The following text reviews our current understanding of genetic and epigenetic factors associated with CKD and the progressive decline in GFR.

**Genetic Risk Factors**

Recent years have seen a substantial shift away from individual gene variant, single-center case-control association studies to more powerful analyses that can robustly identify genetic risk factors for CKD (73, 75, 80). Improvements in laboratory techniques and associated bioinformatics tools have

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*L. J. Smyth and S. Duffy contributed equally to this work.*

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led to cost-effective genotyping approaches, including high-density microarrays examining up to 5 million single-nucleotide polymorphisms (SNPs) and next-generation sequencing approaches that can interrogate an individual’s entire genome (DNA or RNA) for <£1,000 (<$1,500). More detailed phenotypic characterization of CKD as well as adjustment for study-specific factors and extended clinical covariates, such as diabetes, hypertension, and drug regimens, are revealing novel genetic risk factors that have been supported across multiple studied populations (14, 34, 105, 107).

Single gene studies. Single gene studies are often focused on a biological or positional candidate gene based on existing data for the phenotype of interest. These studies are relatively quick and inexpensive to conduct as individuals with CKD compared with control (unaffected individuals) association studies, but as genes do not act in isolation, such studies are limited in the information they provide, and the vast majority have observed no association with CKD. More than 3,000 research papers have been published on the genetics of CKD since the 1980s; however, few of these have been replicated in larger cohorts with more comprehensive genotyping, and conflicting reports of associations are frequent. Nevertheless, candidate gene studies still have an important role in terms of following up genome-wide studies and functional validation; a range of bioinformatic in silico tools has been developed to help inform statistical associations (90). Ideally, published population-based genetic studies should report as much information as possible to enable effective downstream interpretation, reanalysis as new statistical approaches are identified, and informed inclusion in meta-analyses. Standardized guidelines, such as the Strengthening the Reporting of Genetic Association studies, include transparent reporting strategies that include study design, experimental details, explicit quality control, genotype counts, and statistical methods (63). Genome-wide studies and next-generation sequencing provide more comprehensive and unbiased approaches that permit genetic interactions to be considered.

**Genome-wide linkage studies.** Genome-wide linkage studies provide a cost-effective method to consider reasonably large regions of the genome using family-based collections of DNA to track transmission of genetic variants linked with CKD. Twenty-three larger-scale linkage studies have been conducted to identify loci associated with CKD, and these consider individuals of different ethnicities. Such studies published in the last 2 yr or reporting significant linkage scores [log$_{10}$ of odds (LOD) score $>3$, $P \leq 10^{-8}$] are shown in Table 1. Every autosomal (chromosomes 1–22) has been linked to a kidney-related phenotype, but independent replication of linkage studies, where the same marker is used and populations have similar phenotype criteria, is uncommon (76). Many original studies do not reach today’s more stringent levels of quality control and statistical significance. Recently, the Family Investigation of Nephropathy and Diabetes consortium reported linkage of eGFR (calculated using the modification of diet in renal disease formula) to chromosome 20q11 (LOD = 3.3, $P = 4.4 \times 10^{-5}$) in Mexican Americans after genotyping 6,000 SNPs in a multiethnic population of 3,960 participants (125). Park and colleagues (87) examined 1,007 individuals from 73 extended families of Mongolian origin for eGFR (calculated by the modification of diet in renal disease formula) as part of the Gene Discovery for Complex traits in large isolated families of Asians of the Northeast project. Two suggestive linkage regions were revealed (9q21, LOD = 2.8; 15q15, LOD = 2.7) and subsequently supported by family-based association that exceeded genome-wide significance in 722 of these individuals; rs17400257 ($P = 7.2 \times 10^{-5}$) is located near a noncoding RNA (LOC102723989) and the FERM domain containing 3 gene on chromosome 9, whereas rs1153831 ($P = 2.5 \times 10^{-11}$) is situated between a noncoding RNA (LOC102724512) and the solute carrier family 30 (zinc transporter), member 4 gene on chromosome 15.

Of particular note, combined analysis of linkage studies for GFR ($n = 14$), urinary albumin-to-creatinine ratio ($n = 11$), serum creatinine ($n = 11$), and creatinine clearance ($n = 4$) did not identify any genomic region that reached statistical significance (100). A particular challenge for linkage studies is the recruitment of sufficient numbers of related family members to enable genetic risk markers to be tracked through multiple generations or in siblings concordant or discordant for kidney disease. Genome-wide association studies (GWASs) offer improved resolution for genotyping, while the typical collection of unrelated cases and controls is much easier for the common renal phenotypes that appear in adulthood.

**GWASs.** GWASs provide a more powerful approach to detect genetic risk factors for CKD where the risk factors have small or moderate effect sizes (73, 102). GWASs typically genotype millions of SNPs, which are selected as “landmarks” to optimize cost-effective evaluation across the human genome. The genetic contribution to CKD is polygenic, meaning that SNPs in many genes need to be combined to form an effective genetic risk profile to help identify presymptomatic individuals who are at higher risk of progressing to CKD and ESRD or at risk of associated cardiovascular complications. Many genetic loci have been associated with CKD (Table 2), but there is limited overlap between SNPs associated with different measures of renal function, specific primary renal diagnosis, or even progressive kidney fibrosis (which is a common final pathway in the pathogenesis of most chronic
Table 1. Linkage studies reported for renal phenotypes published from 2012 to April 2014 or where LOD ≥ 3 or P ≤ 10^{-8} in reverse chronological order by year

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study</th>
<th>Ethnicity</th>
<th>Phenotype (Maximum Population)</th>
<th>Main Findings</th>
<th>Top-Ranked P Value/LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park et al. (87)</td>
<td>2013</td>
<td>Genome-wide linkage scan</td>
<td>Asian (Mongolian)</td>
<td>eGFR (1,007)</td>
<td>Chr 9q21 rs17400257 Chr 15q15 rs1153831</td>
<td>P = 7.21 × 10^{-9}</td>
</tr>
<tr>
<td>Thameem et al. (125)</td>
<td>2013</td>
<td>Genome-wide linkage scan</td>
<td>African-American American-Indian European-American</td>
<td>eGFR in individuals with DKD (3,960)</td>
<td>All subjects for eGFR rs1339048: cM 44 Chr 10p12 Mexican-American: Chr 20q11</td>
<td>P = 5.5 × 10^{-4}</td>
</tr>
<tr>
<td>Pattaro et al. (91)</td>
<td>2009</td>
<td>Linkage analysis</td>
<td>Mexican-American European (4 isolated villages)</td>
<td>General population for SrCr (2,239)</td>
<td>European-American: Chr 15q12 Chr 7p14, 9p21, 11p15, 15q15–21, 16p13, and 18p11 replicated in at least one population/pooled analysis Novel locus found (Chr 10p11) Chr 22q13 (61 cM) independent of diabetes and hypertension was detected over a region containing the MYH9 gene</td>
<td>LOD = 2.84</td>
</tr>
<tr>
<td>Arar et al. (3)</td>
<td>2008</td>
<td>Genome-wide linkage scan</td>
<td>Mexican-American</td>
<td>uACR, SrCr, CrCl, and eGFR (848 participants from the San Antonio Family Heart Study)</td>
<td>uACR: Chr 20q12 (marker D20S107) SrCr: Chr 9q21 (marker D9S922) CrCl: Chr 2p25 (marker D2S1780) eGFR: Chr 9q21 (marker D9S1122) LOD = 2.94 LOD = 2.62 LOD = 2.05 LOD = 3.87</td>
<td>LOD = 3.4</td>
</tr>
<tr>
<td>Freedman et al. (22)</td>
<td>2008</td>
<td>Genome-wide linkage scan</td>
<td>African-American and Caucasian</td>
<td>Diabetic/hypertensive for GFR and SrCr type 2 diabetes (1,067)</td>
<td>uACR: genomic location cM 28 Chr 2p25 Chr</td>
<td>LOD = 1.61</td>
</tr>
<tr>
<td>Kopp et al. (49)</td>
<td>2008</td>
<td>Linkage analysis</td>
<td>African-American</td>
<td>Focal segmental glomerulosclerosis (412)</td>
<td>Chr 22 Multiple SNPs were recessively associated with focal segmental glomerulosclerosis</td>
<td>LOD = 9.2, peak of 12.4 centered on the MYH9 gene</td>
</tr>
<tr>
<td>Rogus et al. (103)</td>
<td>2008</td>
<td>Genome-wide linkage scan</td>
<td>95% Caucasian</td>
<td>Type 1 diabetes and DKD (100 sib pairs)</td>
<td>rs260462 Chr 19q LOD = 3.1</td>
<td></td>
</tr>
<tr>
<td>Schelling et al. (110)</td>
<td>2008</td>
<td>Genome-wide linkage scan</td>
<td>African-American, American-Indian, European-American, and Mexican-American (majority)</td>
<td>Diabetes for GFR (941)</td>
<td>Highest combined populations: genomic location cM170 Chr 7 Highest overall: Mexican (51.6% total): genomic location cM 170 Chr 7 (markers: D7S3070 and D7S058) LOD = 3.28 LOD = 4.23</td>
<td></td>
</tr>
<tr>
<td>Leon et al. (57)</td>
<td>2007</td>
<td>Genome-wide linkage scan</td>
<td>African-American and Caucasian</td>
<td>Hypertensive for GFR (2,380)</td>
<td>uACR: genomic location cM 12 Chr 19 GFR: genomic location cM 94 Chr 14 LOD = 2.6 LOD = 3.29</td>
<td></td>
</tr>
<tr>
<td>Puppala et al. (97)</td>
<td>2007</td>
<td>Linkage analysis</td>
<td>Mexican-American</td>
<td>GFR (diabetes)</td>
<td>Chr 2q (nearest marker D2S427) LOD = 3.3</td>
<td></td>
</tr>
<tr>
<td>Krowlewski et al. (52)</td>
<td>2006</td>
<td>Genome-wide linkage scan</td>
<td>European-American</td>
<td>Type 2 diabetes (5,656)</td>
<td>Genomic location cM 33 Chr 22 LOD = 3.7</td>
<td></td>
</tr>
<tr>
<td>Placha et al. (96)</td>
<td>2006</td>
<td>Genome-wide linkage scan</td>
<td>European-American</td>
<td>Type 2 diabetes (63 extended families)</td>
<td>Diabetes: genomic location cM 202 Chr 2 (nearest marker: D2S1384) Nondiabetes: genomic location cM 161 Chr 3q (nearest marker: D3S1744) All relative pairs: genomic location cM 23 Chr 7p (nearest marker: D7S3047-D7S3051) LOD = 4.1 LOD = 2.2 LOD = 4.0</td>
<td></td>
</tr>
<tr>
<td>Turner et al. (128)</td>
<td>2006</td>
<td>Genome-wide linkage scan</td>
<td>African-American, White</td>
<td>Hypertensive for GFR (2,372)</td>
<td>Genomic location cM 43 Chr 7 Genomic location cM 221 Chr 3 LOD = 3.65 LOD = 2.21</td>
<td></td>
</tr>
<tr>
<td>Hunt et al. (37)</td>
<td>2004</td>
<td>Genome-wide linkage scan</td>
<td>European-American</td>
<td>General population for SrCr (1,516)</td>
<td>Genomic location cM 145 Chr 2 (nearest marker D2S1334) LOD = 3.15</td>
<td></td>
</tr>
</tbody>
</table>

LOD, log_{10} of odds; eGFR, eGFR, estimated glomerular filtration rate (GFR); DKD, diabetic kidney disease; SrCr, serum creatinine; MYH9, myosin heavy chain 9; uACR, urinary albumin creatinine ratio; CrCl, creatinine clearance.

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Table 2. GWASs and substantial meta-analyses published from 2012 to April 2014 or where \( P \leq 10^{-8}\)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study</th>
<th>Ethnicity</th>
<th>Phenotype (Maximum Population)</th>
<th>Main Findings</th>
<th>Top-Ranked ( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lin et al. (62)</td>
<td>2014</td>
<td>Meta regression analysis for ACE</td>
<td>Asian</td>
<td>CKD (99 populations)</td>
<td>D allele associated with CKD in hypertensive Asian men; no association with DKD</td>
<td>Odds ratio = 3.7, 95% confidence interval: 1.8–7.7</td>
</tr>
<tr>
<td>Olden et al. (82)</td>
<td>2014</td>
<td>GWAS and meta-analysis</td>
<td>European</td>
<td>Urinary UMOD levels (10,884)</td>
<td>rs12917707 (Chr 16, UMOD)</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>Sandholm et al. (105)</td>
<td>2014</td>
<td>GWAS</td>
<td>White</td>
<td>Type 1 diabetes and uACR (1,925 discovery; 3,750 replication)</td>
<td>Five SNPs in GLRA3; discovery rs2410601 (Chr 12, PS3-DH2DA); replication</td>
<td>( P = 5 \times 10^{-8} )</td>
</tr>
<tr>
<td>Yin et al. (141)</td>
<td>2014</td>
<td>Meta-analysis for apolipoprotein E</td>
<td>Chinese Han</td>
<td>T2DKD (4,615 cases; 2,867 controls)</td>
<td>Both apolipoprotein E2 and E4 alleles (Chr19, apolipoprotein E) T allele in the Asian population</td>
<td>( P &lt; 0.01 )</td>
</tr>
<tr>
<td>Zhou et al. (149)</td>
<td>2014</td>
<td>Meta-analysis for TGF-β1</td>
<td>Asian</td>
<td>DKD (2,337 cases; 2,526 controls)</td>
<td>rs1800470 (Chr 19, TGF-β1)</td>
<td>( P = 0.04 ) (overall)</td>
</tr>
<tr>
<td>Sandholm et al. (106)</td>
<td>2013</td>
<td>GWAS; sex specific</td>
<td>Asian, African, White; FinnDiane, Warren 3-GoKinD UK, GENIE consortia</td>
<td>Type 1 diabetes and nephropathy (7,761)</td>
<td>rs4972593 was significantly associated with ESRD in women but not in men (Chr 2, CDCA7 and transcription factor Sp3)</td>
<td>( P &lt; 5 \times 10^{-8} ) (Asian)</td>
</tr>
<tr>
<td>Yamada et al. (140)</td>
<td>2013</td>
<td>GWAS</td>
<td>Japanese</td>
<td>CKD (1,352 cases; 2,499 controls)</td>
<td>rs9846911 (Chr 3, KRT4-III7)</td>
<td>( P = 7 \times 10^{-4} )</td>
</tr>
<tr>
<td>Ding et al. (18)</td>
<td>2012</td>
<td>Meta-analysis for renin-angiotensin-aldosterone genes</td>
<td>Caucasian</td>
<td>T1DKD and T2DKD (4,377 cases and 4,905 controls from 34 case-control studies)</td>
<td>DDK risk for Caucasians compared with both Asians and the dominant model</td>
<td>Odds ratio = 2.11, 95% confidence interval: 1.06–4.23</td>
</tr>
<tr>
<td>Kang et al. (40)</td>
<td>2012</td>
<td>Meta-analysis for receptor for advanced glycation end products</td>
<td>Caucasian</td>
<td>Type 2 diabetes and nephropathy (29 articles)</td>
<td>No significant results found</td>
<td>Not significant</td>
</tr>
<tr>
<td>Kryluk et al. (47)</td>
<td>2012</td>
<td>Meta-analysis</td>
<td>Multiethnic</td>
<td>IgA nephropathy (15,544)</td>
<td>rs6677604 (Chr 1, CFH) rs9275596 (Chr 6, MTCO3P1-DQA2) rs9357155 (Chr 6, PSMB8) rs1883414 (Chr 6, DPB2) rs2412971 (Chr 22, HORMAD2)</td>
<td>( P = 4.6 \times 10^{-13} )</td>
</tr>
<tr>
<td>Lyons et al. (68)</td>
<td>2012</td>
<td>GWAS</td>
<td>Caucasian</td>
<td>ANCA-associated vasculitis (2,687 cases; 7,500 controls)</td>
<td>Anti-proteinase 3 ANCA associated with HLA-DP</td>
<td>( P = 4.6 \times 10^{-13} )</td>
</tr>
<tr>
<td>Niu and Qi (79)</td>
<td>2012</td>
<td>Meta-analysis for methylenetetrahydrofolate reductase</td>
<td>Caucasian, Asian, African, and Latin-American</td>
<td>T1DKD and T2DKD (7,807)</td>
<td>rs1801133 (Chr 1, MTHFR) TT carriers are more likely to develop DKD than individuals with diabetes without DKD and nondiabetic controls</td>
<td>( P = 0.042 ) (compared with diabetic individuals); ( P = 0.01 ) (compared with nondiabetic controls)</td>
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<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study</th>
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<th>Phenotype (Maximum Population)</th>
<th>Main Findings</th>
<th>Top-Ranked (P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okada et al.</td>
<td>2012</td>
<td>Meta-analysis of GWASs</td>
<td>East Asian (18 studies as part of the Asian Genetic Epidemiology Network)</td>
<td>CKD (110,347)</td>
<td>17 new loci associated with kidney function related traits</td>
<td>(P = 4.5 \times 10^{-16})</td>
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<td></td>
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<td>Blood urea nitrogen concentration: (rs10767873) (Chr 11, MPPED2-DCDC5)</td>
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<td>SrCr: (rs10277115) (Chr 7, UNCX-MICALL2)</td>
<td>(P = 4.6 \times 10^{-11})</td>
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<td>eGFR: (rs10277115) (Chr 7, UNCX)</td>
<td>(P = 1 \times 10^{-10})</td>
</tr>
<tr>
<td>Palmer et al.</td>
<td>2012</td>
<td>GWAS</td>
<td>African-American</td>
<td>Type 2 diabetes and ESRD (3,132 cases; 3,317 controls)</td>
<td>rs7560163 [Chr 2 AC14777.4 (LOC)]</td>
<td>(P = 7 \times 10^{-9})</td>
</tr>
<tr>
<td>Pattaro et al.</td>
<td>2012</td>
<td>GWAS</td>
<td>European ancestry</td>
<td>eGFR and CKD (130,600)</td>
<td>Six novel loci in combined analysis associated with eGFR based on SrCr</td>
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<td></td>
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<td></td>
<td>rs3925584 (Chr 11, MPPED2)</td>
<td>(P = 8.4 \times 10^{-18})</td>
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<td></td>
<td>rs6431731 (Chr 2, DDX1)</td>
<td>(P = 4.3 \times 10^{-08})</td>
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<td></td>
<td>rs2453580 (Chr 17, SLC47A1)</td>
<td>(P = 2.1 \times 10^{-09})</td>
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<td></td>
<td>rs12124078 (Chr 1, DNAJC16, CASP9, and AGMAT); age ≥65 yr</td>
<td>(P = 9.0 \times 10^{-13})</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>rs11078903 (Chr 17, CDK12, MED1, FBXL20); age ≥65 yr</td>
<td>(P = 4.0 \times 10^{-08})</td>
</tr>
<tr>
<td>Sandholm et al.</td>
<td>2012</td>
<td>GWAS</td>
<td>White; GENIE consortium</td>
<td>T1DKD (12,564)</td>
<td>Overall analysis detected a significant association between ACE IVD polymorphism and a risk of DKD for all genetic models</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>2012</td>
<td>Meta-analysis for ACE</td>
<td>White</td>
<td>T1DKD and T2DKD (14,108 cases; 12,472 controls)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Asian</td>
<td></td>
<td>Most significant SNP is (rs9275596) (Chr 6, MTC03P1)</td>
<td>(P = 4 \times 10^{-8})</td>
</tr>
<tr>
<td>Williams et al.</td>
<td>2012</td>
<td>Meta-analysis of previously reported associations</td>
<td>White, GENIE consortium</td>
<td>Type 1 diabetes and nephropathy (6,566)</td>
<td>No independent replication, however, fixed-effect meta-analysis retained significance for (rs161740) (Chr 7 EPO)</td>
<td>(P = 2 \times 10^{-9})</td>
</tr>
<tr>
<td>Boger et al.</td>
<td>2011</td>
<td>Meta-analysis</td>
<td>European descent (mix of CKDGen Consortium and CARE Consortium)</td>
<td>Type 1 diabetes and uACR (27,246)</td>
<td>Identified 3 loci in myosin heavy chain and common deletion of (CFHR1 + CFHR3) at Chr1q32 and locus at Chr 22q12</td>
<td>(P = 1.9 \times 10^{-12})</td>
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<tr>
<td></td>
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<td></td>
<td>Follow-up in Chinese and European cohorts</td>
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<tr>
<td>Gharavi et al.</td>
<td>2011</td>
<td>GWAS</td>
<td>Chinese-Han ancestry</td>
<td>IgA nephropathy (1,194 cases; 902 controls)</td>
<td>Follow-up (1,950 cases; 1,920 controls)</td>
<td></td>
</tr>
</tbody>
</table>

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Table 2.—Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study</th>
<th>Ethnicity</th>
<th>Phenotype (Maximum Population)</th>
<th>Main Findings</th>
<th>Top-Ranked ( P ) Value</th>
</tr>
</thead>
</table>
| Liu et al. (64)      | 2011 | GWAS and meta-analysis             | African ancestry,          CArE Renal Consortium | eGFR, CKD, uACR, and microalbuminuria (8,110) | uACR: rs2880072 (Chr 6, \( FNDC1 \))  
\( P = 2.98 \times 10^{-7} \)  
uACR: rs4555246 (Chr 18, \( DOK6 \))  
Microalbuminuria: rs1009840 (Chr 6, \( SGK1 \))  
eGFR SrCr: rs6581768 (Chr 12, \( DYSK2 \))  
eGFR SrCr: rs7111394 (Chr 11, \( KCNQ1 \))  
CKD: rs6428106 (Chr 1, \( RGS2 \)) | \( P = 5.3 \times 10^{-7} \)  
\( P = 1.2 \times 10^{-5} \)  
\( P = 2.62 \times 10^{-6} \)  
\( P = 3.6 \times 10^{-6} \)  
\( P = 3.7 \times 10^{-6} \) |
| Stanescu et al.      | 2011 | GWAS                               | White ancestry (French, Dutch, and British) | Idiopathic membranous nephropathy (556 cases; 556 controls) | rs4664308 (Chr 2q24, Chr 2, \( PLA2R1 \))  
s2187668 (Chr 6p21 Chr 6, \( HLA-DQA1 \)) | \( P = 8.6 \times 10^{-29} \)  
\( P = 8 \times 10^{-93} \) |
| Yu et al. (144)      | 2011 | GWAS                               | Han Chinese                      | IgA nephropathy (1,434 cases and 4,270 controls in discovery; 2,703 cases and 3,646 controls in replication) | rs3803800 (Chr 17p13, \( TNFSF13 \))  
s4227 (Chr 17, \( MPDU1 \) and \( SOX15 \))  
s2738048 (Chr 8, \( DEFA9P-DEFA10P \))  
s660895 (Chr 6, \( DRB1-DQA1 \))  
s1794275 (Chr 6, \( DQB1-MTCO3P1 \))  
s2523946 (Chr 6, \( HCG9 \))  
s12537 (Chr 22, \( MTMR2 \)) | \( P = 9.4 \times 10^{-11} \)  
\( P = 4.3 \times 10^{-10} \)  
\( P = 3.2 \times 10^{-14} \)  
\( P = 4.1 \times 10^{-20} \)  
\( P = 3.4 \times 10^{-13} \)  
\( P = 1.7 \times 10^{-11} \)  
\( P = 1.2 \times 10^{-11} \) |
| Chambers et al.      | 2010 | GWAS                               | European White participants from nine studies | SrCr and CKD (23,812) | rs10206899 (Chr 2, \( ALMS1P \); creatinine)  
rs3127573 (Chr 6, \( SLC22A2 \); creatinine)  
rs8068318 (Chr 17, \( TRX2 \); creatinine)  
rs4805834 (Chr 19, \( CEP89 \); creatinine) | \( P = 1.2 \times 10^{-15} \)  
\( P = 6.5 \times 10^{-10} \)  
\( P = 3.4 \times 10^{-10} \)  
\( P = 4.5 \times 10^{-11} \)  
\( P = 1 \times 10^{-7} \) |
| Feehally et al.      | 2010 | GWAS                               | European ancestry              | IgA nephropathy (244 cases; 4,980 controls) | rs3115573 and rs3130315 (Chr 6p, \( myosin heavy chain \)) | \( P = 1.07 \times 10^{-23} \) |
| Genovese et al.      | 2010 | GWAS                               | African-American                | Focal segmental glomerulosclerosis and hypertensive ESRD (205 cases; 180 controls) | rs73885319 and rs60910145 (Chr 22, \( APOJLI \)) | \( P = 1 \times 10^{-7} \) |
| Kottgen et al.       | 2010 | Meta-analysis of GWASs             | European ancestry              | eGFR, SrCr, serum cystatin C, and CKD (90,075; 67,093 and 22,982 in replication) | eGFR SrCr: rs17319721 (Chr 4, \( SHROOM3 \))  
eGFR SrCr: rs10109414 (Chr 8, \( STC1 \))  
eGFR SrCr: rs2453533 (Chr 15, \( SLC28A2-GATM \))  
eGFR SrCr: rs12917707 (Chr 16, \( UMOD \))  
eGFR SrCr: rs267734 (Chr 1, \( CERS2-ANXA9 \))  
eGFR SrCr: rs1260326 (Chr 2, \( GCKR \))  
eGFR SrCr: rs13538 (Chr 2, \( NAT8 \)) | \( P = 1.1 \times 10^{-19} \)  
\( P = 1 \times 10^{-8} \)  
\( P = 4.6 \times 10^{-22} \)  
\( P = 1.2 \times 10^{-20} \)  
\( P = 1.2 \times 10^{-12} \)  
\( P = 3.0 \times 10^{-14} \)  
\( P = 4.5 \times 10^{-14} \) |
kidney disorders). Genes that have been associated with CKD from GWASs include regulator of G protein signaling 2, myosin heavy chain 9 (MYH9)-apolipoprotein L1 (APOL1), protein kinase, AMP-activated, γ2 noncatalytic subunit (PRKAG2), and uromodulin (UMOD) (42, 50, 51, 64). SNPs in the MYH9-APOL1 gene region (chr 22q13.1) demonstrate significant linkage disequilibrium and have been independently associated with focal segmental glomerulosclerosis (FSGS) (26, 49), diabetic kidney disease (85), and ESRD (23), although not all studies have observed significant associations (74). Intriguingly, it has been observed that APOL1 SNPs associated with kidney disease in are common in African chromosomes but absent from European chromosomes, and this may be because APOL1 variants confer protection against

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>Study</th>
<th>Ethnicity</th>
<th>Phenotype (Maximum Population)</th>
<th>Main Findings</th>
<th>Top-Ranked P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maeda et al.</td>
<td>2010</td>
<td>Gene-based SNPs</td>
<td>Japanese, Singaporean, Korean, and</td>
<td>eGFR SrCr: rs347685 (Chr 3, TFDP2)</td>
<td>P = 3.0 x 10^-11</td>
<td></td>
</tr>
<tr>
<td>(70)</td>
<td></td>
<td></td>
<td>European</td>
<td>eGFR SrCr: rs11959928 (Chr 5, DAB2)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>eGFR SrCr: rs6420094 (Chr 5, SLC34A1)</td>
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<td></td>
<td></td>
<td></td>
<td>eGFR SrCr: rs8831858 (Chr 6, VEGFA)</td>
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<td>eGFR SrCr: rs4744712 (Chr 9, PIP5K1B)</td>
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<td></td>
<td></td>
<td>eGFR SrCr: rs6262777 (Chr 13, DACH1)</td>
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<td></td>
<td>eGFR SrCr: rs1394125 (Chr 15, UBE2Q2)</td>
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<td></td>
<td></td>
<td>eGFR SrCr: rs12460876 (Chr 19, SLC7A9)</td>
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<td>eGFR cystatin C: rs911119 (Chr 20, CST3)</td>
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<td></td>
<td></td>
<td>eGFR cystatin C: rs653178 (Chr 12, ATXN2)</td>
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<td></td>
<td></td>
<td>CKD: rs7805747 (Chr 7, PRKAG2)</td>
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<td></td>
<td></td>
<td></td>
<td>rs2268388 (Chr 12, ACACB)</td>
<td></td>
<td>P = 1.4 x 10^-6</td>
</tr>
</tbody>
</table>

Pattaro et al. (92) 2010 Meta-Analysis of GWAS European Special Population Network project SrCr (4,006. Replication: 2,035) rs4588898 (Chr 8, COL22A1) rs12300068 (Chr 12, STT1) rs4821482 (Chr 22, MYH9) Hypertension: rs4821482 (Chr 22, MYH9) Focal segmental glomerulosclerosis: rs16996674 (Chr 22, MYH9) P = 2.1 x 10^-4

Kao et al. (42) 2009 GWAS African ancestry and European ancestry ESRD (1,372 cases; 806 controls) Nondiabetic ESRD: rs1005570 (Chr 22, MYH9) Hypertension: rs4821482 (Chr 22, MYH9) Focal segmental glomerulosclerosis: rs16996674 (Chr 22, MYH9) P = 5.7 x 10^-5

Kotting et al. (50) 2009 GWAS European ancestry (2,388 CKD cases and 21,466 CKD replication cases) CKD and GFR (19,877) CKD: rs12917707 (Chr 16, UMOD) eGFR SrCr: rs12917707 (Chr 16, UMOD) eGFR SrCr: rs17379721 (Chr 4, SHROOM3) eGFR SrCr: rs2467853 (Chr 15, GATM/SPATA5L1) eGFR cystatinC: rs13038305 (Chr 20, CST3) eGFR cystatinC: rs1731274 (Chr 8, STC1) P = 3.5 x 10^-11
African sleeping sickness (Trypanosomiasis) due to Trypanosoma brucei rhodesiense (25). The UMOD gene is particularly intriguing as rs12917707 has been significantly associated with CKD, eGFR, urinary uromodulin levels, and kidney diseases inherited in a Mendelian manner (19, 50, 51, 64, 82, 95). UMOD is also known as Tamm-Horsfall protein, which is the most abundant urinary protein and provides protection against urinary tract infections. There has been reported overlap between common genetic variants identified for multifactorial diseases and related rare Mendelian disease, but few have been reported for CKD (88).

The majority of genetic associations with CKD are specific to a population with particular ethnicity, for example, the D allele of angiotensin-converting enzyme is associated with hypertensive Asian males (62). Serum/glucocorticoid-regulated kinase 1 is associated with albuminuria in individuals with African ancestry (64), whereas cubilin and the glycline receptor α3-subunit (GLRA3) are associated in European populations (10, 105). Fibronectin type III domain containing 1 and docking protein 6 are associated with the urinary albumin-to-creatinine ratio in individuals of African ancestry. Not unexpectedly, the cystatin C gene on chromosome 20 is associated with GFR estimated using cystatin C levels in European populations, with ataxin 2 also identified for this phenotype (50, 51). Additionally, Alstom syndrome 1 pseudogene, agmatine ureohydrolase, caspase 9, centrosonal protein 89 kDa, ceramide synthase 2-annexin A9, ChaC, cation transport regulator homolog 1, cyclin-dependent kinase 12, collagen type XXII-α1, Dab, mitogen-responsive phosphoprotein, homolog 2, dachshund family transcription factor 1, DEAD (Asp-Glu-Ala-Val) box helicase 1, DnaJ (heat shock protein 40) homolog subfamily C member 16, dual-specificity tyrosine phosphorylation-regulated kinase 2, exonuclease 3'-5' domain containing 1, F-box and leucine-rich repeat protein 20, glycine amido transferase, glucokinase (hexokinase 4) regulator, INOS0 complex subunit, K+ voltage-gated channel KQT-like subfamily member 1, mediator complex subunit 1, MICAL-like 2, metallophosphoesterase domain containing 2, N-acetyltransferase 8, phosphatidylinositol-4-phosphate 5-kinase type 1B, shroom family member 3, solute carrier family 7 (amino acid transporter light chain, bo + system), member 9, solute carrier family 22 (organic cation transporter), member 2, solute carrier family 28 (concentrative nucleoside transporter), member 2, solute carrier family 34 (type II sodium/phosphate cotransporter), member 1, solute carrier family 47 (multidrug and toxin extrusion), member 1, stanniocalcin 1, T-box 2, transcription factor Dp-2, ubiquitin-conjugating enzyme E2Q family member 2, UMOD, UNC homeobox, and vascular endothelial growth factor A are associated with serum creatinine (13, 51, 64, 81, 92, 93).

For specific primary renal diseases, AF4/FMR2 family, member 3, erythropoietin, GLRA3, and repulsive guidance molecule family member A-multiple C2 domains transmembrane 2 genes are associated with renal function in individuals with type 1 diabetes (105, 107, 138). Engagement and cell motility 1 (ELMO1) and acetyl-CoA carboxylase-β are associated with renal disease in individuals with type 2 diabetes, although these do not reach genome-wide significance (70, 114). Multiple genes on chromosome 6 (chromosome 6 open reading frame 10, major histocompatibility complex class II DQ-α2 (HLA-DQA2), DPB2, HLA-DR-β1 (DQB1)-HLA-DQA2, HLA-DQB1-MT-CO3 pseudogene 1, HLA complex group 9, and proteasome (prosome, macropin) subunit B-type 8) are associated with IgA nephropathy as well as complement factor H, TNF (ligand) superfamily member 13, mannan-P-dolichol utilization defect 1, sex-determining region Y-box 15, defen sin-α5, pseudogene-defensin-α5 pseudogene, and HORMA domain containing 2 genes (20, 47, 144). MYH9 and APO11 genes are associated with FSGS and hypertensive nephropathy (26, 42). Idiopathic membranous nephropathy is particularly striking, with two major loci identified from a relatively small case-control association study with French, Dutch, and British participants: rs4664308 in the phospholipase A2 receptor 1 (PLA2R1) gene (P = 8.6 × 10^-29) and rs2187668 in the HLA-DQA1 gene (P = 8 × 10^-93), odds ratio: 78.5, 95% confidence interval: 34.6–178.2 (119). These initial associations have been confirmed in multiple independent populations (12, 16, 66). Antibodies to PLA2R1 are observed in 70% of patients diagnosed with idiopathic membranous nephropathy, with high levels of these antibodies linked to declining renal function (35, 41). Rapidly progressive crescentic glomerulonephritis can occur secondary to systemic vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA). A recent GWAS (68) confirmed that ANCA-associated vasculitis has a genetic component. Anti-proteinase 3 ANCA has been associated with genes encoding HLA-DP (P = 6.2 × 10^-89), α1-antitrypsin (serpin peptidase inhibitor clade A member 1, P = 5.6 × 10^-15), and proteinase 3 (P = 2.6 × 10^-7). Anti-myeloperoxidase ANCA has been associated with HLA-DQ (P = 2.1 × 10^-8) (68). These GWASs confirm that it is feasible to identify genetic risk factors in the causal pathway for CKD.

Gender-specific effects. Men have a higher risk of CKD, there are increased incidence and prevalence of ESRD in men compared with women (78), and there are sex-specific responses to kidney injury (5, 115). Several gender-specific associations with CKD have been reported, most convincingly rs4972593, which is associated with ESRD in women but not in men (106). This SNP is located on chromosome 2q31 between Sp3 transcription factor (SP3) and cell division cycle associated 7 (CDC7) genes, although several pseudogenes also reside in this genomic region. SP3 encodes a transcription factor that stimulates or represses the function of multiple genes and shows sex-specific glomerular expression levels (106, 106), whereas CDC7 is a cell cycle gene that has been associated with the blood pressure response to dietary intervention in the GenSalt GWAS (P = 3.6 × 10^-8) (32).

Meta-analysis. Meta-analysis across multiple independent collections with similar phenotype inclusion and exclusion criteria will help to determine true associations with CKD. More than 250 reports describing meta-analyses have been published for CKD, but challenges persist in terms of genetic or phenotypic heterogeneity, accounting for relevant covariates, insufficient reporting of quality control strategies, small sample sizes, inappropriate cases compared with controls, unclear reporting of significance values, or effect sizes without confidence intervals or adjustment for multiple testing. Catalogues of GWASs have been developed, such as the National Human Genome Research Institute GWAS resource, which includes information from >1,750 manually curated publications for >11,910 SNPs; >10 publications are mapped for CKD, and SNP-CKD association results may be viewed along-
side SNPs associated with other traits of interest, such as diabetes or hypertension (136).

GWASs to date have focused on the “common disease, common variant” hypothesis (54, 102); however, analysis of rare SNPs with a minor allele frequency of <5% are informing CKD research. Multicenter consortia are required to provide adequately powered samples sizes for effective rare variant studies; however, such studies have revealed novel findings for complex disorders such as vascular disease (46, 94, 135), lipid levels (55, 104), and autism (61, 143), with some mega-consortia now exceeding 335,000 individuals (unpublished observations). Analysis of low-frequency variants from the GENIE consortium, where all individuals have type 1 diabetes, revealed different genetic profiles for macroalbuminuria and ESRD, suggesting that although there are contributions from rare variants for both phenotypes, they did not overlap substantially; future study designs should consider albuminuria and ESRD as distinct phenotypes (14). With the exception of the SH2B adaptor protein 3 gene, which is involved in a range of signaling activities and associated with type 1 diabetes, evidence of a shared genetic component between SNPs associated with blood pressure, coronary artery disease, carotid intima-media thickness, pulse wave velocity, and retinal venular caliber have not been observed with GFR or albuminuria (83).

More comprehensive approaches. Detailed analysis facilitated by whole exome, next-generation sequencing resources has revealed a missense mutation in the anillin gene associated with FSGS (24) as well as olfactory receptor family 2 subfamily L member 13 (P = 6.2 × 10^-5) and APOL1 (P = 4.6 × 10^-5) genes nominally associated with diabetic and non-diabetic kidney disease in African-Americans (17). A number of population-based studies have aimed to whole genome sequence several thousand individuals and link this information to detailed medical records, for example, the United Kingdom’s 100K genome project (www.genomicsengland.co.uk).

Other large-scale resources include the United States Genetic Epidemiology Research on Aging cohort, which links genome-wide genetic data to medical information for >78,000 individuals. There are, of course, a myriad of ethical and legal issues associated with making these large-scale data sets publicly available, but such resources offer tremendous potential to identify genetic risk factors and illustrate the biology underlying CKD.

The many genetic associations described above highlight that identifying underlying genetic mechanisms for CKD remains challenging. Heritability estimates (131), a measure of the contribution of genetic effects in a population, for CKD vary between 20% and 80%, and interindividual differences in CKD appear to be due to genetic factors (38, 56, 69, 86). However, despite notable successes for kidney disease, much of this heritability remains unexplained. “CKD” represents many complex underlying phenotypes, and it is apparent that a detailed characterization of cohorts and carefully defined phenotypes that are harmonized across recruitment centers are critically important. Higher-density genotyping arrays and next-generation sequencing, along with carefully defined phenotypes, will help delineate the complex genetic factors associated with CKD. Ongoing, more comprehensive genetic studies for CKD may elucidate clinically relevant genetic risk profiles, but epigenetic phenomena are being increasingly recognized as playing an important role in the pathogenesis of CKD (Fig. 2).

Epigenetic Risk Factors

Large-scale GWASs have improved our understanding of the genetic basis of CKD, but incorporating epigenetic features associated with CKD may further improve our understanding of genetic architecture and disease biology. Epigenetics refers to heritable (transgenerational) or dynamic changes in gene expression resulting from factors other than a direct change in

![Fig. 2. Genetic and epigenetic developments for CKD. miRNA, microRNA; EWAS, epigenome-wide association study; DKD, diabetic kidney disease; GWAS, genome-wide association study.](http://ajprenal.physiology.org/)

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the DNA sequence of the genome. These modifications may be inherited or occur in response to current or past environmental exposures such as diet, drugs, or illness. Extreme environmental stress, e.g., intrauterine growth retardation secondary to maternal starvation, may lead to long-term epigenetic modification of the genome with an increased lifetime risk of diabetes and cardiovascular disease (39). Altered metabolic states, such as hyperglycemia in diabetes or uremia in CKD, may lead to changes in gene expression mediated by epigenetic mechanisms, and this has been referred to as hyperglycemic or uremic “memory” (30, 45, 72). Epigenetic features are attractive therapeutic targets as they can be altered and may be distinct to individual cell types, thus offering potential for drugs targeting epigenetic changes in specific tissues. For example, treatment of db/db mice with losartan reversed most of the epigenetic changes associated with diabetic kidney disease and ameliorated the disease in this model (101).

Similar to the technological advances that have helped develop GWASs and next-generation sequencing, it is now cost effective and practical to conduct epigenome-wide association studies (EWASs) for CKD (Fig. 3) (8, 72, 75). This field is still in its infancy, with <700 publications returned in PubMed from searching “epigenetic” and “kidney” (May 2014), but research is progressing quickly, with more than one-third of these papers published in the past 2 yr. The majority of association data for CKD is available for three key epigenetic features: chromatin modifications, RNA interference (RNAi), and DNA methylation. These epigenetic mechanisms do interact, adding further complexity as to how they are implicated in gene regulation.

Chromatin modification. The broadest level of complex epigenetic control occurs at histones. Histones package DNA into chromatin and help control the accessibility of DNA to the transcriptional machinery, thereby influencing gene expression. Common types of histone posttranslational modification include acetylation, methylation, phosphorylation, and ubiquitination, which result in open and transcriptionally active, or closed and transcriptionally inactive, states of chromatin. Importantly, global chromatin changes may not reflect local changes; for example, hyperacetylation typically leads to transcriptionally active DNA ( euchromatin), but it may also repress the transcription of local genes (2). Acetylation and deacetylation of histone lysine residues are regulated inversely by the actions of a family of enzymes called histone acetyltransferases and histone deacetylases (HDACs), whereas methylation of lysine residues occurs by methyltransferases. Methylated histones can occur as single, di-, and trimethylated residues, each having a different effect on expression. Histone-based epigenetic regulation for transcription activation or silencing typically depends on enzymes, which are themselves amenable to therapeutic intervention; >20 HDAC inhibitors are under development for clinical use, and several have received Federal Drug Administration approval (124, 137). One such drug is vorinostat, which received Federal Drug Administration approval for the treatment of cancer (http://www.cancer.gov/cancertopics/druginfo/fda-vorinostat), but this drug also demonstrates renoprotective properties (1, 147). Histone modification has an important role altering histone methylation in the transforming growth factor (TGF)-β pathway, which has previously been established as an important mechanism of fibrosis in CKD; treatment of rat mesangial cells with a TGF-β antibody reversed hyperglycemia-induced changes associated with diabetic kidney disease, leading to a reduction in the expression of these fibrotic genes (65, 120, 145). Evidence of the modifiable nature of epigenetic marks by currently licensed drugs suggests promising clinical applications for CKD.

RNAi. RNAi represents a more refined level of gene expression control by impeding the efficiency of translation of mRNA to protein. Many noncoding RNAs exist, but the most extensively studied are microRNAs (miRNA) and long noncoding RNAs. miRNAs are a class of small noncoding RNAs (~22 nucleotides in length) that act as intrinsic regulators of gene expression in a variety of biological processes that influence CKD (109, 111). As the name suggests, long noncoding RNAs are longer nonprotein coding RNAs with important roles in genomic imprinting, chromosome X inactivation, gene expression, and regulation of protein activity (31). RNAi has a key role in both kidney development, kidney fibrosis, and homeostasis; for example, when dicer, an enzyme central in the production of miRNAs, was inactivated in mouse podocytes, it led to renal failure and death (77, 89, 113). Skewing of female X chromosome inactivation has been associated with kidney fibrosis, progressive renal disease, and kidney transplant outcomes (117, 148). As with histone modifications, many kidney-miRNA studies have focused on fibrosis via the TGF-β pathway and limiting or reversing fibrotic damage through this mechanism (11, 43, 123, 132). For example, miRNA-192 has been identified as a key regulator of collagen formation in diabetic kidney disease mouse models (44), whereas in human renal biopsies from patients with diabetic kidney disease, TGF-β upregulated miRNA-192 expression in proximal tubule cells, correlating with fibrosis and reduction in eGFR (53). Inhibition of kidney miRNA-192 was associated with decreased renal fibrosis and reduced proteinuria in a diabetic kidney disease mouse model (98). The defined influence of miRNAs in kidney damage and their ready accessibility in blood and urine make them attractive biomarkers or targets for therapeutic intervention (99); miRNAs involved in kidney disease are shown in Table 3.

DNA methylation. DNA methylation is a commonly studied epigenetic modification for complex diseases such as CKD (9, 48, 72, 75, 108, 118, 139, 142, 146). DNA methylation refers to the covalent addition of a methyl group to cytosine by DNA methyltransferases at CpG sites. Typically, DNA methylation is associated with gene silencing, however, it can also activate genes if the methylation directly inhibits cofactors or miRNA that would normally repress transcription. Population-based studies for DNA methylation have been facilitated by the development of commercial arrays that enable low-cost EWASs to be performed with single CpG site resolution. The largest EWAS performed for CKD to date examined 485,577 unique methylation sites in 407 individuals, revealing blood-derived differential methylation associated with CKD in cutlike homeobox 1, ELMO1, FK506-binding protein 5, inhibin-βA-AS1, protein tyrosine phosphatase receptor type N polypeptide 2, and PRKAG2 genes; two-thirds of top-ranked differentially methylated sites were also reflected in kidney tissue, and several genes were supported by analysis of gene expression (118). Of particular interest, PRKAG2 has been associated with CKD by genomic DNA SNP studies (51) as well as DNA methylation and gene expression data (118).
Most recently, in the CRIC study, Wing and colleagues (139) compared 20 individuals with the fastest rate of eGFR decline ($-5.1 \pm 1.2 \text{ ml}-\text{min}^{-1}\cdot\text{m}^{-2}$) with 20 participants with improvement in GFR during follow-up ($2.2 \pm 1.4 \text{ ml}-\text{min}^{-1}\cdot\text{m}^{-2}$), revealing hypermethylation of nephrocystin-4-like protein, IQ motif and Sec7 domain 1, and transcription factor 3 in the group with relatively stable renal function, along with differential methylation of nitric oxide synthase 3, NF-$\kappa$ light polypeptide gene enhancer in B cells inhibitor-like 2, clusterin, NF-$\kappa$ light polypeptide gene enhancer in B cells inhibitor-$\beta$, TGF-$\beta_3$, and TGF-$\beta_1$ genes (139). Table 4 shows larger-scale studies for DNA methylation associated with CKD. Similar to chromatin modification and RNAi, DNA methylation is also potentially inducible or reversible; for

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**Fig. 3.** Annotated chromosome map highlighting the location of epigenetic features associated with CKD. EWAS publications are highlighted by color: Smyth et al. (118) in red, Sapienza et al. (108) in green, Wing et al. (139) in yellow, Ko et al. in purple (48), and Bell et al. (9) in blue.
### Table 3. miRNAs associated with kidney pathologies

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Pathologies/Sample</th>
<th>Main Outcome</th>
<th>P Value</th>
</tr>
</thead>
</table>
| Krupa et al. (53)                 | 2010 | 22 renal biopsies from DKD patients divided into 9 progressors, 9 nonprogressors, and 4 late presenters | Expression of 365 miRNAs assessed  
Reduced miRNA-192 expression and reduction in eGFR  
Reduced miRNA-192 expression and fibrosis score | $P < 0.001$ |
| Lv et al. (67)                    | 2013 | Urine samples from 32 patients with previously characterized fibrosis              | Levels of urinary miRNA-29 and miRNA-200 families were assessed. Reduced levels of all members (miRNA-29a, miRNA-29b, miRNA-29c, miRNA-200b, and miRNA-200c) correlated with CKD  
Levels of miRNA-29c alone correlated with both eGFR and fibrosis | $P < 0.05$ |
| Szeto et al. (121)                | 2012 | Urine sample of 56 CKD patients                                                   | Eight miRNAs were assessed;  
miRNA-21 and miRNA216a expression correlated with renal function decline. Other miRNAs (miRNA-15, miRNA-7, miRNA-192, miRNA-216a, and miRNA-217) showed significant correlation within diagnosis groups and renal function | $P = 0.026; P < 0.001$ |
| Argyropoulos et al. (4)           | 2013 | Urine samples of 40 type 1 diabetes patients;  
10 did not progress to DKD  
10 patients developed DKD  
10 patients with intermittent microalbuminuria  
10 patients with persistent microalbuminuria | A total of 27 miRNAs were found to be present at significantly different levels in different stages of untreated nephropathy (including miRNA-221-3p, miRNA-619, miRNA-486-3p, miRNA-335-5p, miRNA-552, miRNA-1912, miRNA-1224-3p, miRNA-424-5p, miRNA-141-3p, and miRNA-141-3p). Pathway analysis revealed association with genes involved in growth factor signaling and renal fibrosis previously implicated in DKD | $P < 0.05$ |
| Ramachandran et al. (99)          | 2013 | 98 patients with AKI                                                               | Assessed 7 miRNAs selected from a pilot study on 12 patients. Four were associated:  
miRNA-21  
miRNA-200c  
miRNA-423  
miRNA-4640  
miRNA-21 and miRNA-200c have previously been linked to CKD | $P = 0.005$ |
| Wang et al. (134)                 | 2010 | Kidney biopsies of 43 patients with IgA nephropathy and 20 controls               | Expression of miRNA-200, miRNA-205, and miRNA-192 were assessed in each biopsy.  
There was decreased miRNA-200c expression in IgA nephropathy as opposed to the control group  
Conversely, higher levels of expression were reported in IgA nephropathy patients for:  
miRNA-141  
miRNA-205  
miRNA-192 | $P = 0.007$  
$P = 0.017$  
$P = 0.033$  
$P = 0.027$ |

*miRNAs, microRNA; AKI, acute kidney injury.*
### Table 4. Summary of EWASs for populations affected with CKD

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Study Type</th>
<th>Ethnicity</th>
<th>Kidney Disease (Population)</th>
<th>Outcome</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smyth et al. (118)</td>
<td>2014</td>
<td>EWAS; Infinium HumanMethylation 450 K BeadChip</td>
<td>Caucasian (UK)</td>
<td>CKD (255 cases; 152 controls)</td>
<td>Statistically significant association for methylation in 399 genes. Strong candidates for kidney disease with one or more CpG site include: CUX1, CpG site: cg02385808, ELMO1, CpG site: cg08044454, FKBP5, CpG site: cg00130530, INHBA-AS1, CpG site: cg26478599, PTTPRN2, CpG site: cg24701780, PRKAG2, CpG site: cg10370262</td>
<td>$P = 3.67 \times 10^{-9}$</td>
</tr>
<tr>
<td>Wing et al. (139)</td>
<td>2014</td>
<td>EWAS; Infinium HumanMethylation 450 K BeadChip</td>
<td>African-American or non-Hispanic</td>
<td>CKD (20 cases; 20 controls)</td>
<td>Hypermethylation of key kidney genes in the “stable kidney function” group compared with the “rapid progression group.” Genes involved in the promotion of epithelial-to mesenchymal-transition and renal fibrosis include: NPHP4, CpG site: cg14260998, IQSEC1, CpG site: cg09601150, TCF3, CpG site: cg13685746, TCF3, CpG site: cg14403762</td>
<td>Other CKD-related genes with significant differential methylation include NO33, NFKBIL2, CLU, NFKBIB, TGFBI, and TGFBI; $P = 7.8 \times 10^{-5}$</td>
</tr>
<tr>
<td>Ko et al. (48)</td>
<td>2013</td>
<td>EWAS</td>
<td>Mixed, Asian and Pacific Islander</td>
<td>CKD/renal Fibrosis (12 cases; 14 controls in discovery; 21 DKD; 66 controls for validation)</td>
<td>4,751 differentially methylated regions between control and diseased tubule samples, 1,535 (1,061 of which overlapping in validation data) unique genes in the vicinity of the differentially methylated regions enriched for genes for cell adhesion and development related functions including: collagen, fibronectin, TGF-β</td>
<td>$P \leq 0.01$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Validation by Infinium HumanMethylation 450 K BeadChip</td>
<td>White non-Hispanic Black and non-Hispanic</td>
<td></td>
<td>450K arrays from 66 control and 21 DKD microdissected kidney samples where $P \leq 10^{-10}$, COLAA3, KCNQ1, STK10, TMPRSS4, KANK2, RIN3, MBP, and INPP5A</td>
<td>$P = 10^{-10}$</td>
</tr>
<tr>
<td>Hsu et al. (36)</td>
<td>2012</td>
<td>EWAS; MethylAmp Global DNA Methylation Quantification Ultra Kit</td>
<td>Unknown</td>
<td>CKD/hemodialysis (20 cases; 20 controls)</td>
<td>Global DNA methylation amounts were not significantly different between normal subjects and hemodialysis patients</td>
<td>$P &gt; 0.05$</td>
</tr>
<tr>
<td>Zawada et al. (146)</td>
<td>2012</td>
<td>SuperTAG methylation-specific digital karyotyping</td>
<td></td>
<td>CKD/hemodialysis (10 cases; 10 controls)</td>
<td>4,288 genomic loci with differential DNA methylation; 149 genes associated with proatherogenic and inflammatory processes</td>
<td>$P = &lt;10^{-10}$</td>
</tr>
<tr>
<td>Sapienza et al. (108)</td>
<td>2012</td>
<td>EWAS; Illumina HumanMethylation27 BeadChip array</td>
<td>African-American and Hispanic</td>
<td>Diabetes and ESRD (24 cases; 24 controls)</td>
<td>187 genes differentially methylated between cases and controls; 39 of which have been previously associated with kidney disease. These were involved in pathways including inflammation, oxidative stress, and fibrosis. Genes of particular interest included: MYL9, MMP10, TIMP4, and MTHFR</td>
<td>A “DiffScore” of $&lt;-20$ or $&gt;+20$</td>
</tr>
<tr>
<td>Bell et al. (9)</td>
<td>2010</td>
<td>EWAS; Illumina HumanMethylation27 BeadChip array</td>
<td>Caucasian (UK/Ireland)</td>
<td>T1DKD (96 cases; 96 controls)</td>
<td>19 unique CpGs identified with an association with DKD, including a gene previously associated with T1DKD: UNC13B, CpG site: cg07341907</td>
<td>$P = 3.1 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

EWAS, epigenome-wide association studies.
example, RAS protein activator-like 1 hypermethylation contributes to kidney fibrosis (7), but this pathological hypermethylation was reversed by Tet3-mediated hydroxymethylation in cell culture, kidney biopsies, and a mouse model, suggesting that endogenous demethylating agents may be transiently activated as a novel therapy for CKD (33, 122). Several epigenetic features are strong candidates for novel, minimally invasive biomarkers to aid diagnosis of kidney complications and the prediction of individuals at high risk of developing CKD, whether using whole blood or in silico techniques to adjust for different cell fractions. The identification of epigenetic marks in kidney tissue associated with CKD will increase understanding of the biological mechanisms underlying CKD, but the cellular heterogeneity of the kidney and the invasive nature of collecting a kidney biopsy limit its usefulness for routine clinical use. Next-generation sequencing approaches are also helping refine epigenetic risk factors, and data integration projects may lead to the identification of a clinically useful genetic-epigenetic-metabolomic-transcriptomic risk profile (6, 15, 116).

Conclusions

In summary, CKD is a significant public health concern with increasing numbers of individuals affected worldwide. There is a need to identify biological markers that help identify individuals who are at higher risk of developing CKD so that targeted therapies can be used to delay the onset or progression of CKD. It is equally important to identify individuals at low risk for CKD so that these persons can avoid exposure to unnecessary drug treatment and finite healthcare resources are optimally used. Personalized medicine needs robust tools to enhance risk estimation for CKD by integrating clinical, molecular, and environmental data. Significant progress is being made identifying genetic and epigenetic risk factors associated with CKD. Research has been accelerated by the creation of multicenter consortia genotyping larger populations with carefully defined harmonised phenotypes, advanced statistical methods for handling large-scale data sets, and longitudinal studies with extensive follow-up to analyze preclinical and clinically relevant factors for CKD. The enhanced personalization of treatment for individuals at high risk of developing CKD would enhance patient care, facilitate optimization of therapies, potentially reduce healthcare costs, thereby decreasing the economic burden from CKD, and enhance the efficiency of clinical trials by permitting the selection of appropriate populations stratified by genetic risk and predicted response to drugs.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


36. Fox CS, Matsushita K, Woodward M, Bilo HJ, Chalmers J, Heer-


