Unraveling the genetics of chronic kidney disease using animal models

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CHRONIC KIDNEY DISEASE IS a significant medical problem in the United States, affecting roughly 25 million people. The annual cost associated with the treatment of end-stage renal disease is estimated at $22 billion, with an additional economic burden associated with monitoring and treating patients with earlier stages of kidney disease (41). Chronic kidney disease also contributes indirect medical costs because it is a risk factor for cardiovascular disease, including myocardial infarction, atherosclerosis, stroke, and hypertension (21, 28, 33, 36). Mortality from myocardial infarction, stroke, and coronary artery disease escalates with increasing urinary albumin levels (25, 32), and the link between chronic kidney disease and cardiovascular disease is supported by numerous other studies (6, 8, 14, 16, 19, 20, 23). The number of patients currently undergoing treatment for end-stage renal disease is ~400,000, but this number is projected to soar to 2.2 million by 2030, primarily due to the growth of the number of diabetic patients (41). Therefore, understanding the genetic components of chronic kidney disease onset and progression could lead to improved diagnosis and treatment of both renal and cardiovascular disease.

The National Kidney Foundation defines chronic kidney disease according to the presence or absence of kidney damage and the level of kidney function, regardless of the type (clinical diagnosis) of kidney disease (28). The primary measure of kidney function is glomerular filtration rate, which is often estimated as creatinine clearance from serum and urine creatinine concentrations (28). In studies of chronic kidney disease using animal models, serum creatinine concentrations are used to assess kidney function because glomerular filtration rate cannot be easily measured or reliably estimated in animal models. The principal marker of kidney damage is increased urinary protein excretion, either total urinary protein or specifically urinary albumin (28). Assessment of proteinuria or albuminuria is useful in both clinical and experimental studies of chronic kidney disease.

KIDNEY DISEASE LOCI IN HUMANS

Bowden (3) recently reviewed the current research in the genetics of kidney disease in humans. He described that the identification of genes involved in common forms of kidney disease has not been very successful thus far. Except for some monogenic forms of kidney disease (e.g., polycystic kidney disease, congenital nephrotic syndrome, focal segmental glomerulosclerosis), very few causal genes have been identified. This is probably because kidney disease is a polygenic disease with strong environmental factors. The progressive stages of chronic kidney disease also deter the identification of causal genes due to phenotypic heterogeneity. The National Kidney Foundation has outlined four classes of risk factors for adverse outcomes associated with chronic kidney disease: 1) increased susceptibility to kidney damage; 2) direct initiation of kidney damage; 3) progression of renal failure after initiation of renal damage; and 4) complications of end-stage renal disease (28). Thus a precise clinical diagnosis is critical to identify genetic risk factors for each class in humans.

Association studies, which test whether one allele of a gene is more common in patients with kidney disease than in controls, are commonly used to test the role of specific candidate genes in human kidney disease. This candidate gene

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approach has identified several genes associated with renal disease, such as the genes encoding angiotensin-converting enzyme (ACE) and apolipoprotein E (APOE). However, association studies have problems with replication, power, and the use of different statistical analysis methods (3). Also, testing specific candidate genes in association studies requires a priori knowledge of the candidate gene, so this method will not identify novel genes affecting kidney disease.

Another approach for finding disease genes in humans is through genome-wide analysis, which requires no a priori knowledge of a candidate gene and can identify novel genes underlying kidney disease. Linkage studies examine the whole genome by testing for coinheritance of chromosomal regions with disease in families. Although linkage studies have successfully identified kidney disease loci in humans, detecting linkage for a polygenic disease with a moderate relative risk requires a large number of sibling pairs, and genetic heterogeneity further complicates genetic analysis of the trait. Identifying the causal genes underlying these loci is difficult and expensive.

An alternative method of genome-wide analysis is linkage disequilibrium (LD) mapping, which tests unrelated affected individuals and controls for differences in the frequency of genetic variants throughout the genome. LD mapping using single-nucleotide polymorphisms (SNPs) offers new potential for mapping kidney disease genes in humans. However, a useful level of LD is unlikely to exceed an average distance of 3 kb in the general population, which implies that ∼500,000 SNPs will be required for whole genome studies (22). Although this approach would probably result in much higher resolution in genome scans, widespread application of LD mapping does not seem feasible until cost-effective, high-volume SNP genotyping is available.

Recently, concordance in quantitative trait loci (QTL) among species was shown for several complex traits, including hypertension among rats, mice, and humans (38, 39), arthritis between rats and humans (13), and HDL cholesterol levels between mice and humans (42). Concordance of QTL among species suggests that common disease genes underlie these QTL and implies that animal models, in which gene identification is simpler, can be used to find conserved disease genes in humans. Therefore, we reviewed the literature on genetic studies using animal models of chronic kidney disease and examined the concordance between chronic kidney disease QTL across species to determine the relevance of genetic studies in animal models to kidney disease in humans.

KIDNEY DISEASE LOCI IN THE RAT

The rat has served as the principal animal model for investigating the genetics of chronic kidney disease. Several rat strains that exhibit kidney disease have been crossed with resistant strains to identify kidney disease QTL (summarized in Table 1). In one study, fawn-hooded hypertensive (FHH) × August-Copenhagen-Irish (ACI)F1 animals were backcrossed to FHH, and the progeny were phenotyped for several biochemical markers of renal impairment (4). Brown et al. (4) found a clear linkage with chromosome 1 for plasma creatinine, albumin, and urea levels, proteinuria, and kidney lesions and named this QTL Rf-1. This locus accounts for 37% of the total variance in proteinuria and 39% of the total variance in the kidney lesions in this cross. Rf-1 was recently confirmed in a congenic strain in which the whole QTL interval from FHH was introgressed into the ACI genetic background (30). A second locus (Rf-2) on chromosome 1, proximal from Rf-1, has a significant effect on plasma albumin, plasma creatinine, and kidney lesions. In contrast to Rf-1, Rf-2 is also linked to blood pressure (4). In addition to Rf-1 and Rf-2, three other QTL for proteinuria were found in an intercross between the same inbred strains (37); Rf-3, Rf-4, and Rf-5 were mapped to chromosomes 3, 14, and 17, respectively.

Shiozawa et al. (37) also described complex interactions between the Rf loci. Because the products of interacting genes are likely to interact biologically (e.g., receptor-ligand, heterodimers), detecting gene interactions is important in revealing the underlying genetic network and allows the construction of new hypotheses about the pathway (Korstanje R, Li R, Stylianov J, Sheehan S, Paigen B, and Churchill G, unpublished observations). Shiozawa et al. (37) report the interaction of Rf-1 with every other Rf locus and between Rf-2 and Rf-3, Rf-2 and Rf-4, Rf-3 and Rf-4, Rf-3 and Rf-5, as well as Rf-4 and Rf-5. With the biological interaction kept in mind, identifying the gene underlying Rf-1 will facilitate the identification of the interacting genes.

Schulz and co-workers (34) used three different crosses to identify QTL involved in kidney disease. The first cross, in which F1 animals from a cross between the Munich-Wistar-Fromter (MWF) rat and the Lewis (LEW) rat were backcrossed to MWF rats, was phenotyped for urinary albumin excretion (UAE) and glomerulosclerosis. No QTL were found for glomerulosclerosis, but four significant QTL were detected for UAE on chromosomes 1, 5, 12, and 17 (34). The QTL on chromosome 1 overlaps Rf-2 (4), and the chromosome 17 QTL overlaps Rf-5 (37). Consomic strains in which either the SBH/y chromosome 1 or 17 was introgressed onto the SBN/y genetic background confirm the presence of proteinuria QTL on these chromosomes (44). Their second cross was an intercross between Dahl salt-sensitive (SS) rats and spontaneously hypertensive rats (SHR). They identified seven suggestive or significant UAE QTL on chromosomes 2, 6, 8–11, and 19 (29). Only the QTL on chromosome 6, which overlapped a suggestive QTL from the (MWF × LEW)F1 × MWF backcross, was found previously. This (SS × SHR)F2 intercross confirmed all the QTL from an independent study in which (SS × SHR)F1 animals were backcrossed to SS (12). The backcross also detected additional QTL for proteinuria and albuminuria on chromosomes 1 and 13. Because SS contributed recessive susceptibility alleles at both loci, the backcross to SS had greater power to detect these QTL. The presence of an SS allele responsible for proteinuria on chromosome 13 was already shown by Cowley et al. (5) using consomic rats. In a third cross, they used two strains from their first two crosses and backcrossed (MWF × SHR)F1 animals to MWF (35). Significant linkage for albuminuria was found with loci on chromosomes 1, 6, 8, and 9 (35), all confirming QTL previously found in other crosses.

Finally, one QTL on the proximal end of chromosome 13 for proteinuria was reported in a [Buffalo (BUF)/Mna × WKY/
are concordant with kidney disease QTL identified in Pima Indians (18). The albuminuria QTL on rat chromosome 11 is concordant with two QTL for creatinine clearance on human chromosome 3q27 found in African-Americans and Caucasian-Americans (7). The albuminuria QTL on rat chromosome 13 is concordant with a systemic lupus erythematosus glomerulonephritis susceptibility locus (Sle1) in mice (26) and a monogenic form of familial focal segmental glomerulosclerosis in humans (43). The QTL for albuminuria on rat chromosome 19 is concordant with the mouse region containing the Os mutation, which causes glomerular hypertrophy, severe glomerulosclerosis, and a 50% reduction in nephron number in mice (15).

The finding that kidney disease QTL in rat are concordant with kidney disease loci in mice and humans suggests that conserved disease genes may underlie these QTL. This implies that kidney disease QTL in animal models can predict the locations of disease genes in humans. For example, after Rf-1 was identified, two groups tested the homologous human region in an African-American population (11) and in Utah pedigrees (17). The region was significantly linked to end-stage renal disease in both populations, supporting the concept that comparative genomics can identify conserved loci under-
lying kidney disease. However, additional investigation is required to verify that the same causal genes account for QTL conserved across species.

ONSET AND PROGRESSION: DIFFERENT GENES?

Kidney disease caused by hypertension, diabetes, or atherosclerosis could have different pathogenic mechanisms. However, it is striking that some loci found in different disease populations are concordant. For example, $Rf-1$ found in hypertensive rats was detected in separate cohorts of diabetic and atherosclerotic patients, supporting the idea that this locus causes renal impairment independently of elevated blood pressure (4). This observation implies that $Rf-1$ promotes kidney disease progression regardless of the disease initiating kidney damage.

Additional evidence that $Rf-1$ contributes to kidney disease progression, rather than onset, comes from examining the time course of kidney disease. Although SS rats exhibit significant proteinuria at 8 wk old, $Rf-1$ does not contribute to the phenotype until 12 wk (12). This explains why $Rf-1$ was not detected in 8-wk-old (SS × SHR)F$_2$ males (29). In contrast to $Rf-1$, the kidney disease locus on chromosome 6 is present at 8 wk and becomes progressively stronger over time (12, 29, 34, 35), suggesting that this QTL participates in both kidney disease onset and progression.

THE MOUSE AS A GENETIC MODEL FOR KIDNEY DISEASE

Forward genetic approaches (transgenic and knockout studies) using the mouse have been very successful in investigating the biological function of genes and testing mechanisms of kidney disease. However, the mouse has been underutilized for reverse genetic approaches (mutagenesis and QTL analysis) to identify novel genes causing kidney disease. The exceptional genetic tools available for the mouse, such as the genetic and technical infrastructure to support both high-throughput mutagenesis and QTL analysis, make it a good model system for the genetic analysis of chronic kidney disease.
Mutagenesis, including chemical mutagenesis and gene trapping, is an effective method for identifying genes underlying disease (2, 31, 40). Because mutagenesis induces random mutations throughout the genome, mutagenesis could potentially identify all the genes underlying a phenotype. Mutagenesis has been used successfully to identify a gene defect causing kidney disease. *Neph1*, a component of the glomerular-slit diaphragm, was identified in a gene-trap mutagenesis screen in mouse ES cells (10) based on its homology to NEPHRIN, a recently discovered component of the glomerular-slit diaphragm. *Neph1*-deficient mice exhibit podocyte foot-process effacement and severe proteinuria (10). However, this strategy required previous knowledge of kidney disease genes to identify *Neph1* based on homology. We and others are currently screening randomly mutagenized mice for kidney disease, which may uncover novel genes and biological pathways regulating kidney function independently of our current knowledge. These novel mutant strains may also provide new animal models for the study of kidney disease.

Although mutagenesis can detect genes involved in kidney disease, QTL analysis is more likely to identify the subset of genes underlying common forms of kidney disease in the general population. Thus far, QTL analysis to uncover kidney disease loci in mice has been mostly limited to detecting QTL containing genes modifying monogenic disease models, such as Alport’s syndrome (1) or polycystic kidney disease. The mouse is a very good model organism for QTL analysis to identify primary genetic determinants of kidney disease because of the genetic tools available for identifying causal genes underlying QTL. These tools include consomic and recombinant inbred strain sets, the nearly complete genomic sequence of four inbred strains, a forthcoming haplotype map of common inbred mouse strains, and the availability of genetic engineering technologies. We are conducting a survey of kidney disease phenotypes among 48 common inbred mouse strains to facilitate choosing strains for QTL analysis in mice. Knowing which kidney disease QTL are conserved between mice, rats, and humans will prioritize QTL for further investigation, with those conserved among all three species being the highest priority. Also, conserved QTL can be narrowed through comparative genomics.

CONCLUSIONS
Finding kidney disease genes in humans is hampered by genetic complexity and high costs. Genetic studies in rats have already identified many QTL. The repeated findings of QTL in the same region in different crosses suggest that there are several key loci involved in kidney disease. So far, genetic studies investigating kidney disease loci in mice have been limited, but both mutagenesis and QTL analysis studies are currently in progress. With our current knowledge, concordance between rat and human loci suggests conservation of important kidney disease genes. This concordance validates the use of animal models to search for these genes. Animal models (rat and mouse in particular) can make identifying candidate genes easier because of less genetic complexity, better genetic tools, and a controlled environment. Combining the knowledge obtained in the three different species is a powerful way to unravel the genetics of chronic kidney disease.

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